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Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus**

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Abstract

During the course of its embryonic development, the European lobster *Homarus gammarus* LINNAEUS exhibits progressive increases in content of water (54.0 to 83.1%), ash (2.7 to 16.7%), protein (47.4 to 50.9%) and non-protein nitrogen (1.0 to 2.4%), and steady decreases in content of fat (43.8 to 25.4%) and energy (6343 to 5431 cal/g dry organic substance). Cumulative yolk utilization efficiency during the total development is 81.8% for dry weight; the corresponding value for energy is 60.1, for protein 75.6 and for fat 47.4%. Energy content of a single egg is 10.49 cal. Of the 4.20 cal expended for metabolic processes of the embryo, only 13.3% energy is drawn from protein oxidation; fat oxidation supplies as much as 87.7% energy, that of carbohydrate only 2.3%. Embryonic development results in a remarkable decrease in net yolk utilization efficiency, which falls from 85.5% in the early developmental stages, to less than 70% in later developmental stages. The mean dry weight of a single egg membrane increases from 38 µg (2.2% of egg weight) in a freshly laid and attached egg, to 81 µg in an egg with an almost completely developed embryo. This result supports the earlier observation of CHEUNG (1966) that the formation of the inner chitinous egg membrane occurs after the egg is laid and attached to the setum. Protein seems to be the major constituent of the egg membrane (4049 cal/g dry weight), which has the following composition: protein 70.4%, non-protein nitrogen 0.13%, ash 2.83%. Initial permeability of the egg membrane to water (about 6% of the total water requirement is let in) is followed by a period during which the egg membrane is almost impermeable to water (stages I to III); the egg membrane becomes permeable to water again and lets in 85% of the total water requirement (the rest, i.e. 9%, is metabolic water) at a relatively advanced stage of development. These assumed changes in egg membrane permeability appear to be indicative of variations in the egg's osmoconcentration leading to shiftings in net transport of water. Rates of water and salt uptake during embryonic development are essentially parallel (Fig. 1). The egg membrane remains permeable to salts throughout development; salt intake almost doubles after the egg passes through stage III. A single egg, weighing 3.7 mg requires 4.9 mg water for successful completion of embryonic development. The imbibition of water by the developing marine demersal egg seems to (1) serve in osmotic hatching; (2) float the hatched larva by means of specific gravity reduction; (3) aid the larva to quickly adjust its body temperature. The simple osmotic hatching mechanism, proposed by previous workers, seems to be inadequate to account for the events and timing of the hatching process in the lobster. It is suggested that hatching time is determined not solely by increased internal pressure caused by inflow of water and salts, but also by some unknown internal factor. In the lobster egg, as well as in many other marine demersal eggs, protein metabolism is suppressed to a considerable extent, and fat metabolism is "geared up". Thus, the non-cleidoic lobster eggs exhibit metabolic properties which are typical of cleidoic eggs. This finding is discussed in the light of NEEDHAM's (1950) concept of "cleidoic-terrestrial and non cleidoic-aquatic eggs".

Introduction

This paper is based on publications dealing with the utilization of matter and energy in developing eggs of marine thermo-conformers, especially the crustaceans *Crangon crangon*, *Homarus americanus*, *Ligia oceanica* (PANDIAN, 1967, 1970a, b), and *Eupagurus bernhardus* (PANDIAN and SCHUMANN, 1967); the gastropod *Crepidula fornicata* (PANDIAN, 1969); and the fish *Solea solea* (FLÜCHTER and PANDIAN, 1968). It has been shown that fat and ash are the two important variables during embryonic development of marine demersal eggs; ash content increases from its initial value of 2 to 4% in fresh eggs, to about 15% in freshly hatched larvae; fat decreases during these stages from about 30 to 35% initially, to 15 to 20%; such heavy fat depletion is reflected by calorific content, which decreases from about 6300 to 5200 cal/g dry organic substance. Cumulative yolk utilization efficiency of the total embryonic development is about 60%, and fat oxidation is the main metabolic energy source of the embryo.

The present paper reports on changes in chemical composition and calorific content of the developing eggs and embryos of the European lobster *Homarus gammarus*. It also deals with water balance, hatching mechanism, permeability and composition of egg membrane, as well as net yolk utilization efficiency of lobster embryos at different developmental stages.

* Dedicated to Professor P. KOCHUKUTTA MENON, Montreal, on his 62nd birthday, June 3, 1970.

Material and methods

Material

The European lobster *Homarus gammarus* LINNAEUS (Nephropsidae: Macrura) is said to attain sexual maturity at the age of 10 years. Spawning commences in July and lasts until October, with peak spawning in August. The female carries the fertilized eggs, which number about 13,000 to 15,000. Hatching occurs in the following July, i.e. after a period of some 10 months incubation. For further details on lobster bionomics consult APPELLÖF (1909).

P. JATZKE of the Biologische Anstalt Helgoland, who specializes in lobster ecology, caught the lobsters used in the present investigation near Helgoland (southern North Sea) during his frequent skin diving activities. On three consecutive dates — 6, 7, and 9 July, 1967, — he was extremely fortunate in catching 3 females carrying freshly fertilized eggs. These females were kept individually in large cement aquaria (200 l capacity) in running sea water (31‰ S) at a water temperature of 16° ± 1 °C. The female lobsters were fed crabs and small fishes.

The following arbitrary developmental stages were chosen for chemical analyses:

Stage I: Undeveloped eggs and cleavage stages; average diameter 1.8 mm; dark green; round to oval.

Stage II: Oval eggs; average diameter 1.8 mm; disc-like, milky green-blue embryo spot visible on the animal pole of the dark green egg.

Stage III: Almost half of the egg scarlet-red, the other half dark green; the red-colored part of the egg contains a well formed embryo with dark eye-spots; average diameter of egg 2.2 mm.

Stage IV: Orange-yellow colored egg; oval; diameter 3.0 mm. Embryo fills up the entire egg; eyes oval, black; appendages well developed; heart pumping.

Stage V: Freshly hatched, young lobster larva.

The embryonic development of lobsters has been subdivided into 9 readily distinguishable stages by FIGUEIREDO and BARRACA (1963), and these stages are widely used by lobster biologists (DUNTHORN, 1967). The first 4 stages distinguished in the present investigation correspond to FIGUEIREDO and BARRACA's stages 1, 2, 5, and 9, respectively.

Methods

One hundred to 200 eggs or larvae of each of the above-mentioned stages were washed free from adhering sea water by exposing them twice to distilled water, 45 sec each time. After blotting, the test material was weighed in a Sartorius balance (Type 2604; sensitivity 10 µg).

Water content was determined by weighing the test material before and after drying at 80 °C for 5 h.

Ash content was determined by incinerating the test material (about 50 mg dry substance) in a muffle furnace at 560 °C for a period of 5 h and weighing the residue, as recommended by PAINE (1964).

Protein and non-protein nitrogen contents were determined, following the standard procedure of the micro-Kjeldhal method described by ROTH (1958). Protein content was precipitated by grinding the sample (about 20 mg dry substance) with 0.5 ml of cold trichloroacetic acid in a glass mortar, and subsequently centrifuged. The supernatant contained non-protein nitrogen, and the precipitate protein nitrogen. Protein content was estimated as albumin equivalent by estimating the nitrogen and multiplying the values obtained by 6.25.

Fat content was estimated as the difference between dry weight (about 40 mg dry substance) and fat-free dry weight of the test substance, determined after 6 to 8 h extraction with chloroform-methanol mixture (2:1) in a semi-micro Soxhlet apparatus.

Calorific content was determined with a Parr 1412 semi-micro bomb calorimeter. Sixty to 100 mg dry test substance was generally used for each determination. As the sample available was less than 15 mg in some cases, a known amount of benzoic acid was added as trigger substance.

Table 1. *Homarus gammarus. Estimations of water content in different developmental stages*

Developmental stage	No. of estimates	Mean water content (%)	Standard deviation	Coefficient of variation (%)
Stage I (egg)	9	54.0	± 4.1	7.6
Stage II	3	56.0	± 1.1	2.0
Stage III	4	56.7	± 0.5	0.9
Stage IV	4	72.3	± 1.1	1.5
Stage V (larva)	6	83.1	± 1.1	1.3

Results

Changes in chemical composition

Water content of fresh eggs (stage I) averaged 54.0%, and increased to 83.1% in freshly hatched *Homarus gammarus* larvae (stage V). Table 1 presents the average changes in water content of fresh eggs during the ensuing developmental stages. During the early stages (stages II and III), water content shows little change, and remains around 55%. Subsequently, it increases to 72.3% in stage IV and to 83.1% in stage V.

Except in stage I, deviation from mean water content in all other 4 stages, i.e. stages II to V, was less than 2%, indicating little variability among the different egg samples collected from the 3 mother animals, as well as a high degree of accuracy with which water content can be estimated. Similarly accurate estimations were not possible in eggs of other decapod crustaceans such as *Crangon crangon* (PANDIAN, 1967) and *Eupagurus bernhardus* (PANDIAN and SCHUMANN, 1967), although these were also blotted as carefully as in the case of the eggs of *Homarus gammarus*. The eggs of *C. crangon* and *E. bernhardus* are too small and soft, and retain varying quantities of water on their surfaces and between neighbouring eggs, allowing only crude estimates, which give values of considerable variation.

Table 2. *Homarus gammarus. Changes in chemical composition of developing eggs and freshly hatched larvae. Percentage values, based on dry weights. Brackets indicate the number of estimates made*

Developmental stage	Ash (%)	Protein (%)	Non protein nitrogen (%)	Fat (%)	Carbohydrate (%)
Stage I (egg)	2.7 (3)	47.4 (6)	1.0 (3)	43.8 (3)	5.1
Stage II	4.2 (3)	48.0 (4)	1.4 (3)	44.2 (1)	2.2
Stage III	4.8 (3)	48.9 (3)	1.6 (3)	41.4 (2)	3.3
Stage IV	7.2 (3)	50.4 (3)	2.0 (2)	35.8 (2)	4.6
Stage V (larva)	16.7 (3)	50.9 (6)	2.4 (3)	25.4 (3)	4.6

The average value of 3 water-content estimates of fresh eggs (stage I) from the first female, was 50.5%; it was as high as 52.9 and 58.6% in fresh eggs from the second and third females, respectively. The first female's eggs revealed about 8 blastomeres; the eggs of the second female were approaching blastula stage, and those of the third female were in the post-blastula stage. It is, therefore, possible that the lobster egg actively absorbs water and increases its water content from about 50% when just fertilized, to about 59% when it attains the gastrula stage. Furthermore, the wide deviation (7.6%) from the mean water content (54.0%) of egg stage I seems to be due to the considerable variations among eggs belonging to different early blastula stages (all grouped here under egg stage I). During the course of development, the magnitude of these variations appears to be reduced, as the water content values obtained for all subsequent egg stages (collected from the 3 females) deviates less than 2% from their respective means (Table 1).

Changes in chemical composition of the 5 developmental stages are listed in Table 2. Ash content, only 2.7% in fresh eggs, increases to 16.7% in stage V. Like water, ash increases more markedly in later stages (7.2% stage IV, 16.7% stage V) than in earlier stages (4.2 and 4.8% in stages II and III, respectively).

Ash from all first 3 stages is either greyish black or grey, even after 5 h incineration at 560 °C, while that of stages IV and V is milky white. The egg, either fully or partially dark green in color in the early stages I to III, turns red in stage IV. KÜHN and SØRENSEN (1938a, b) and STERN and SALOMON (1938) have shown that the green pigment ovoverdin, present in fresh eggs of the Canadian lobster *Homarus americanus*, is an astaxanthin-protein complex, which denatures during development to liberate free astaxanthin; during this process, the eggs change color from green to red. It is not clear whether denaturation, leading to a change in salt composition of the developing lobster egg, causes the change in ash color. The liberation of free astaxanthin, i.e. the changing of the partially dark green egg (stage III) to the completely red egg (stage IV), however, seems to mark a very important milestone in the embryonic development of the lobster; for it is then that the egg membrane becomes again permeable to water and a large quantity of water (85% of the total water requirement) is taken up, that salt absorption accelerates, and that the embryo begins to rely heavily upon fat oxidation to meet its metabolic energy demands (Table 5).

Protein content shows relatively little change; it is 47.4% in fresh eggs and increases gradually to 50.9% in freshly hatched larvae (Table 2). During corresponding stages, non-protein nitrogen content increases from 1.0 to 2.4%; in other words, it is 17 µg in a single egg (stage I) and 33 µg in a single larva (Table 5); PANDIAN (1970c) found that as much as 35% of the net increase in non-protein nitrogen is due to chitin synthesis during the development of the lobster. Chitin content of the freshly hatched larva of *Homarus gammarus* is 4.78% of its (dry) body weight, or 81.25 µg (PANDIAN, 1970c). Chitin, a non-protein substance, contains 6.9% nitrogen (RICHARDS, 1951), and hence the non-protein nitrogen moiety of the chitin fraction of a single lobster larva would be 5.6 µg, or 35% of the net increase (16 µg) in non-protein nitrogen content during development.

Fat content of fresh eggs is relatively high (43.8%); an initial increase to 44.2% in stage II is followed by continuous depletion of fat content throughout subsequent embryonic development, especially from stage IV (35.8%) to stage V (25.4%).

Carbohydrate was not directly estimated. Since ash, protein, non-protein nitrogen and fat contents are known, carbohydrate was calculated. It decreases from 5.1% in stage I to 2.2% in stage II, indicating that carbohydrate serves as energy source during early developmental stages. Subsequently, it increases

to 4.6% in stage IV, and maintains this level in stage V (Table 2).

Table 3 shows the changes in calorific content of lobster eggs during the various developmental stages. Calorific content is 6172 cal/g dry weight of the freshly laid eggs (stage I); an initial increase to 6227 cal/g dry weight of stage II eggs is followed by a decrease in calorific content throughout the ensuing developmental stages, especially from 5552 cal/g dry weight in stage IV to 4524 cal/g dry weight in stage V. In general, the trend in calorific content changes parallels that of fat changes.

The number of estimates made to determine the mean calorific content of stages I to V, and their respective variation ranges, are also given in Table 3. Except in stage V, the deviation range from the mean calorific content of all the first 4 stages, i.e. stages I to IV, is less than 3%, while that of freshly hatched larvae is 8.4%, or nearly 3 times more than that of the other stages. This suggests a greater variability among the different samples of larvae used in the calorific determinations. It has been reported by APPELLÖF (1909), and also observed in the present investigation, that the female lobster releases 1,000 to 1,500 larvae per night (around 10 p.m.) for a period of 7 to 10 days. At the beginning of our experiment, larvae were collected for analyses without making any distinction between those hatched on the first, second, or third day, or on subsequent days. It was realized much later that larvae hatched on subsequent days — though possessing more or less the same (dry) weight — contain fewer calories either per individual or per unit weight, than those hatched on the first, second, or third day.

It is of interest here to refer to the observation of PANDIAN (1970a) on the significance of hatching time in the Canadian lobster *Homarus americanus*. This lobster also hatches at the rate of about 1,500 larvae per night (around 10 p.m.) for 4 to 5 days. The larvae which hatched on the first, second and fourth day, were separately subjected to dry weight, ash, and calorific content analyses. The larvae which hatched on the first day contained 4656 cal/g dry weight, while those hatched on the second and fourth days contained 4384 and 3837 cal/g dry weight, respectively; the mean calorific value of these larvae amounted to 4293 ± 417 cal/g dry weight, the deviation range being 9.7% of the mean value. The deviation range from the mean ash content is also similar.

Since, in *Homarus gammarus*, hatching lasts for more than 7 days, the larvae of the European lobster also seem to exhibit considerable variations in dry weight, ash and calorific contents; such variations appear to be reflected in the wide deviation range (8.4%; Table 3) from the mean calorific value (4524 ± 381 cal/g dry weight) of the larvae studied in the present investigation. The significance of hatching time in some decapods has been discussed by the author elsewhere (PANDIAN, 1970a).

Table 3. *Homarus gammarus. 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 (%)	Energy content (cal/g dry organic substance)
Stage I (egg)	6	6172 ± 144	2.3	6343
Stage II	4	6227 ± 147	2.4	6497
Stage III	4	6181 ± 169	2.7	6494
Stage IV	3	5552 ± 148	2.7	5984
Stage V (larva)	8	4524 ± 381	8.4	5431

Table 4. *Homarus gammarus. Dry weight estimations for different developmental stages*

Developmental stage	No. of estimates	No. of eggs or larvae	Mean dry weight of 1 egg or larva (mg)	Coefficient of variation (%)
Stage I (egg)	9	525	1.70 ±0.05	3.0
Stage II	3	150	1.65 ± 0.03	1.8
Stage III	4	200	1.57 ± 0.03	2.0
Stage IV	4	200	1.54 ± 0.02	1.2
Stage V (larva)	6	400	1.39 ± 0.05	3.6

Yolk utilization

Changes in a single developing egg

Results presented in the preceding section show only changes in chemical composition and calorific content of the developing eggs per unit weight. To understand more about the embryonic growth and metabolism, it is necessary that these data are related to the dry weight of a single egg. Table 4 gives the mean dry weight of a single, freshly laid egg, and the changes in its dry weight during subsequent embryonic development. The mean dry weight of an egg decreases from 1.70 mg in stage I to 1.54 mg in stage IV. The mean dry weight of a lobster larva is 1.39 mg; thus, the total loss amounts to 0.31 mg during the whole embryonic development. Of this, a major portion is oxidized to meet the energy requirements of embryonic

metabolism; the remainder (the egg membrane) is sloughed off during hatching. As may be seen from the ensuing section, the mean dry weight of a single egg membrane of egg stage IV is 0.081 mg, equivalent to 0.33 cal (Table 9). Therefore, the actual amount of yolk substance oxidized by embryonic metabolism is 0.229 mg.

Cumulative yolk utilization efficiency

From the values presented in Tables 1 to 4, average changes in chemical composition and calorific content of a single egg from stage I to stage V have been calculated; the values obtained are shown in Table 5. The course of embryonic development exhibits a steady and progressive increase in water content (1.99 to 6.87 mg), ash (0.05 to 0.23 mg) and non-protein nitrogen (0.02 to 0.03 mg), and a continuous decrease in other contents, e. g. protein (0.81 to 0.71 mg), fat (0.745 to 0.35 mg). The remarkable decrease in fat content is reflected in the heavy depletion of the energy content of the egg, which decreases from 10.49 cal in the fresh egg to 6.29 cal in the larva.

Table 5. *Homarus gammarus. Average changes in chemical composition and calorific content of a single egg. All weights given in mg. (Data based on Tables 1 to 4)*

Parameter	Stage I (egg)	Stage II	Stage III	Stage IV	Stage V (larva)
Live weight	3.69	3.75	3.62	5.55	8.26
Water	1.99	2.10	2.05	4.01	6.87
Dry weight	1.70	1.65	1.57	1.54	1.39
Ash	0.046	0.069	0.075	0.112	0.232
Organic substance	1.654	1.581	1.495	1.428	1.158
Protein	0.806	0.792	0.768	0.776	0.707
Non-protein nitrogen	0.017	0.023	0.025	0.030	0.033
Fat	0.745	0.729	0.650	0.551	0.353
Carbohydrate	0.087	0.036	0.052	0.071	0.064
Energy (cal/egg)	10.49	10.28	9.71	8.55	6.29

The cumulative yolk utilization efficiency of the total development for dry weight is 81.8%; the corresponding values are 70.0, 60.1, 87.7, 47.4 and 73.2% for organic substance, energy, protein, fat and carbohydrate, respectively. The differences in these values show that the efficiency with which different substances of yolk are utilized, varies considerably.

During embryonic development, about 4.2 cal are expended on metabolic processes (Table 5). Of this amount, the oxidation of protein (99 µg × 5650 cal/g dry weight = 0.559 cal) and of carbohydrate (23 µg × 4150 cal/g dry weight = 0.095 cal) contributes 13.3% and 2.3%, respectively, while fat oxidation (392 µg × 9400 cal/g dry weight = 3.684 cal) supplies as much as 87.7% energy. Thus, fat oxidation is the main energy source for the embryonic metabolism of the European lobster *Homarus gammarus*.

Net yolk utilization efficiency

Hitherto, cumulative yolk utilization efficiency of the total development (from egg to larva) has been reported. Following GRAY (1926), I have also focussed attention on yolk utilization efficiency of lobster embryos of different stages of development. Since the ratio "body formed/body formed + yolk used for metabolism" is mostly used as a measure of developmental efficiency at any one embryonic stage (GRAY, 1928), it is necessary to know the dry weight and energy content of a single embryo.

We dissected the embryo of *Homarus gammarus* under a microscope; the dissection can be fairly accurate, as the developing embryo can be seen distinctly, milky or creamy-white in color, amidst orange-yellow and/or dark-green yolk. A single embryo of stage II weighs 138 ± 22.1 µg (Table 6; the 16% coefficient of variation is mostly due to the difficulty in dissecting small and delicate embryos at this early stage). The mean dry weight of a single embryo increases to 462 and 826 µg in stages III and IV, respectively.

Since the total dry weight of the dissected embryos yielded only a small amount of test material for bomb calorimetry, I had to use, in almost all cases, about 50 mg benzoic acid (pellet purchased from Parr Instrument Co.; 6318 cal/g). As can be seen from the coefficients of variation (Table 7), I obtained reliable values.

Table 7 shows that embryonic development results not only in a progressive increase in embryo size, but also in an accumulation of more energy per unit body weight. Thus, the mean calorific value of the early embryo, which is 4712 ± 288/cal/g dry weight, in-

creases to about 5400 cal/g dry weight in embryos of stages III and IV.

From values shown in Tables 6 and 7, the energy content of a single embryo at different developmental stages was calculated. A single embryo of stage II contains 650 cal; the energy content progressively increases to 2532 and 4392 cal in embryos of stages III and IV, respectively. Yolk utilization efficiency of the embryo, which is 75.6% in stage II and 76.4% in stage III, decreases to 69.4% in stage IV. It takes about 7 to 10 days for embryos of stage IV to hatch.

Table 6. *Homarus gammarus. Dry weight estimates of embryos of different developmental stages*

Developmental stage	No. of embryos	No. of estimates	Mean dry weight of a single embryo (µg)	Coefficient of variation (%)
Stage II	600	3	138 ± 22.1	16.0
Stage III	400	5	462 ± 38.6	8.4
Stage IV	400	5	826 ± 58.6	7.1
Stage V (larva)	400	6	1390 ± 50.0	3.6

Table 7. *Homarus gammarus. Changes in calorific content of embryos of different developmental stages*

Developmental stage	No. of estimates	Mean calorific content (cal/g dry weight)	Coefficient of variation (%)
Stage II	3	4712 ± 288	6.1
Stage III	3	5481 ± 93	1.7
Stage IV	3	5317 ± 32	0.6
Stage V (larva)	8	4524 ± 381	8.4

During this period, a marked increase in water uptake occurs, which is associated with a period of pronounced cellular differentiation and metabolic activity; the water replaces organic materials used up and constitutes increasing proportions of the embryo itself. Therefore, it is to be expected that the net yolk utilization efficiency is much less than 60% during this stage. GRAY (1928) reported about 56% net efficiency for the embryo of the trout *Salmo fario*, when approaching hatching stage. On the whole, the net yolk utilization efficiency in *Homarus gammarus* decreases from 85.5% to less than 60% during embryonic development. GRAY (1928) also concluded that "the efficiency of development falls from about 85 to 56% as incubation proceeds and no single figure holds good over more than any limited period". Similar observations have been reported for other fishes by IVLEV (1939), SMITH (1957), and LASKER (1962).

Studies on egg membrane

Composition and calorific content

During eclosion, the egg membrane is suddenly ruptured, and sloughed off from the larva. The matter and energy contained in the egg membrane are used neither by the larva nor the embryo. A preliminary attempt has been made to estimate the weight and composition of the egg membrane. Table 8 presents the mean dry weight of a single egg membrane of a fresh egg (stage I) and its weight changes during the subsequent developmental stages.

Table 8. *Homarus gammarus. Estimations of dry weight of single egg membranes in developing eggs*

Developmental stage	No. of eggs	No. of estimates	Mean dry weight of a single egg membrane (µg)
Stage I (egg)	600	3	38 ± 10.8
Stage II	—	—	—
Stage III	400	2	68
Stage IV	450	3	81 ± 14.5
Stage V (larva)	—	—	—

Table 9. *Homarus gammarus. Composition and calorific content of the membrane of the developing egg. Stage IV, i.e. eggs which are approaching hatching in about 10 days*

Number of determinations	Parameter	Values
3	Dry weight	81 µg/egg membrane
1	Protein[a]	70.4%
1	Non protein nitrogen[a]	0.13%
2	Ash	2.83%
2	Energy	4049 cal/g dry weight or 0.33 cal/egg membrane

[a] Herr R. VON HENTIG (Biologische Anstalt Helgoland) estimated and communicated to me these two values.

It may first be stated that the egg membranes are extremely thin and delicate; while peeling them from

the yolk, in some cases part or all of the membrane was lost and, consequently, the values obtained show considerable variations. The dry weight of a single egg membrane (stage I) is 38 μg, or 2.2% of the dry weight of the whole egg. Quite unexpectedly, the dry weight of egg membrane increases to 68 μg (4.3% of egg weight) in stage III and to 81 μg (5.3% of egg weight) in egg stage IV.

Table 9 shows the values obtained for dry weight, ash, and energy content of the egg membrane belonging to egg stage IV. To study the chemical composition, only egg membranes peeled from eggs of stage IV were used, as this was considered important for an understanding of the mechanism of egg hatching. Calorific contents of the egg membranes average 4049 cal/g dry weight, or 4168 cal/g dry organic substance. A single protein estimate made, indicates that protein is the major constituent of the egg membrane (protein 70.4%).

Changes in permeability

I have not made any direct study of the changes in permeability of egg membranes during embryonic development. Since I have data on the quantitative changes in salt (ash) and water contents, it is possible to conclude from the results, possible changes in permeability of the membrane of the developing egg.

Water metabolism: A single lobster egg (3.69 mg wet weight) requires as much as 4.88 mg water for the successful completion of embryonic development. Fertilization and subsequent egg laying seem to be followed by a change in egg membrane permeability to water; a fertilized egg, which contains about 50% water (1.7 mg water/egg) actively absorbs water and increases its water content to about 59% (about 2.0 mg water/egg) when it attains the gastrula stage (p. 156). Subsequently, there is a little change in water content until the developing egg attains stage III (water content 2.05 mg/egg; Fig. 1), indicating that the egg membrane is now impermeable to water, or that its interior is completely isosmotic to the ambient medium. This condition is followed by a period during which the rate of net water intake increases, so that the water content of an egg almost doubles (4.01 mg water/egg, or 72.3% of its wet weight) when development has progressed to stage IV, and more than trebles (6.87 mg water/larva; 83.1% of its wet weight) in the larva (stage V). On the whole, the egg membrane permits about 6% (0.3 mg/egg) of the total water requirement to enter immediately after the egg is fertilized and laid. It is then more or less impermeable to water until stage III; subsequently, it becomes again permeable to water from stages III to V, a relatively short period, during which as much as 85% (4.4 mg/egg) of the total water requirement is absorbed. These assumed changes in egg membrane permeability appear to be indicative of variations in the egg's osmoconcentration leading to shifting in the net transport of water.

The increase in water content of the lobster egg is not solely due to water intake through the egg membrane. A certain amount of water (about 9% of the total requirement), known as metabolic water, is produced through oxidation of yolk substances inside the developing egg. Theoretically, upon oxidation, 1 g fat releases 1.07 g water; 1 g carbohydrate, 0.555 g water; and 1 g protein, 0.41 g water (BALDWIN, 1964, p. 52). Oxidation of 392 μg fat (420 μg metabolic water), 23 μg carbohydrate (13 μg metabolic water), and 99 μg protein (40 μg metabolic water), during the total embryonic development of the lobster (Table 5),

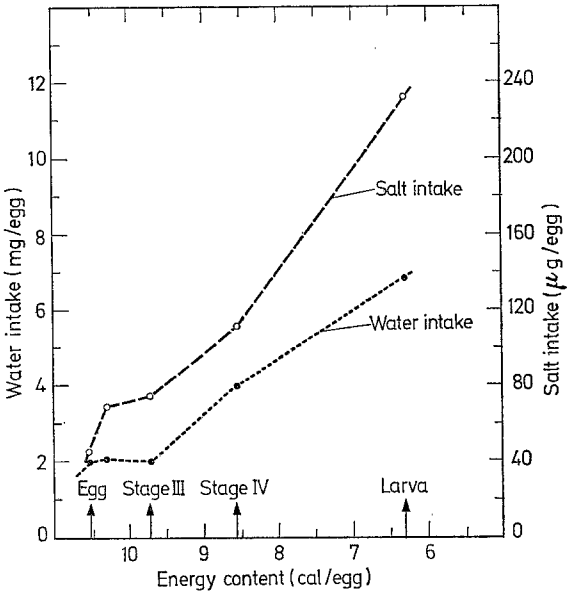

Fig. 1. *Homarus gammarus*. A single developing egg. Water and salt contents as a function of incubation period (expressed in calorific content of the developing egg)

might release about 0.473 mg metabolic water inside the egg. Of the total water requirement of a lobster egg (4.88 mg water/egg), only 9.35% is made available to the embryo through oxidation of yolk substances.

Salt metabolism: Salts seem to be continuously taken up throughout embryonic development. Salt content is nearly doubled (from 0.046 mg/egg in stage I to 0.075 mg/egg in stage III) during the early period when the egg membrane appears to be impermeable to water (Fig. 1). From stage III on, intake rates of salts and water increase; on completion of development, water content has increased nearly $2^1/_2$ times, salt content more than 4 times.

Discussion

The results presented bring out several interesting aspects in regard to the ecophysiology of embryonic

metabolism and growth of the European lobster *Homarus gammarus*. However, I shall restrict the discussion to the following aspects: (1) Ecophysiological consequences of water imbibition, (2) composition and changes in egg membrane weight, (3) egg membrane permeability to water and salt, (4) hatching mechanism, and (5) cleidoic properties of marine demersal eggs.

Ecophysiological consequences of water imbibition

During the embryonic development of *Homarus gammarus*, water content increases from 54.0% in fresh eggs to 83.1% in larvae; i.e. a single egg (3.69 mg wet weight) requires as much as 4.88 mg water for the successful completion of embryonic development. Like any non-cleidoic egg, marine demersal lobster eggs heavily depend upon the environment for water. Evidence of the water requirements of non-cleidoic eggs of freshwater animals has been plentiful, and has been reviewed from time to time by NEEDHAM (1931, 1950). The corresponding data available for marine organisms are summarized in Table 10. Marine demersal eggs of crustaceans and cephalopods increase their water content from about 50—60% to about 70—80% during embryonic development. Such imbibition of water may offer one or more of the following ecophysiological advantages to the egg and/or the larva:

(a) The intake of salt into the developing egg increases the internal osmotic pressure; at critical level, which is synchronized with the completion of embryonic development, hatching is effected. Absorption of salt (and water), and the consequent increase in osmotic pressure represents the hatching mechanism, e.g. in copepods (MARSHALL and ORR, 1954); or one of the hatching mechanisms, e.g. in *Homarus gammarus* (this paper and PANDIAN, 1970a).

Table 10. *Water content changes in developing eggs of marine animals*

Species	Water content (%)		Reference
	Egg	Larva	
Marine demersal eggs which release planktonic larvae			
Squid *Sepia officinalis*	52.5	75.8	RANZI (1930)
Barnacle *Balanus balanoides*	72.6	88.3	BARNES (1965)[a]
Barnacle *Balanus balanus*	78.2	89.4	BARNES (1965)[a]
Shrimp *Crangon crangon*	68.5	87.3	PANDIAN (1967)
Lobster *Homarus gammarus*	54.0	83.1	PANDIAN (this paper)
Lobster *Homarus americanus*	56.2	86.8	PANDIAN (1970a)
Hippa *Emerita analoga*	63.3	83.7	NEEDHAM and NEEDHAM (1930)
Crab *Maia squinada*	56.4	—	NEEDHAM (1931, p. 887)
Hermit crab *Eupagurus* sp.	45.6	77.1	PANDIAN and SCHUMANN (1967)
Average	60.8	84.0	
Marine planktonic eggs which release planktonic larvae			
Sardine *Sardinops caerulea*	88.0	91.2	LASKER (1962)
Plaice *Pleuronectes platessa*	93.0	93.7	NEEDHAM (1931, p. 1114)
Cod *Gadus morhua*	94.7	93.6	NEEDHAM (1931, p. 1114)
Cod *Gadus morhua*	88.0	—	MENGI (1965)
Sole *Solea solea*	90.8	88.9	FLÜCHTER and PANDIAN (1968)
Average	90.9	91.8	

[a] Values from BARNES (1965; Fig. 1 and Tables 2, 3, 8 and 9); the larva values pertain to eggs just prior to hatching or to BARNES' H-stage.

(b) Accumulation of water decreases the specific gravity of developing eggs; attached eggs, carried by the female (in crustaceans) or glued to seaweeds (in cephalopods), release planktonic (free floating) larvae upon hatching. Planktonic eggs release larvae with similarly high water contents (about 91%). Since pertinent data for crustaceans are wanting, data on water contents of planktonic eggs and larvae of some fishes are given (Table 10). Reduction of specific gravity has been successfully employed by these planktonic eggs (larvae) in order to allow floating near the sea surface (see also ALLEE and SCHMIDT, 1957, p. 274).

(c) The high water content seems to offer yet another advantage to these planktonic eggs and larvae as regards thermal adjustment of their bodies. Planktonic organisms may be subjected to quicker temperature changes than demersal forms (diurnal fluctuations in temperature may range to 4 °C in the upper water layers, but only 0.1 °C in depths of 15 to 70 m; CLARKE, 1967); furthermore, due to waves and horizontal currents, planktonic forms may be transported within a short time to distant areas, where temperature conditions may be very different.

Planktonic eggs and larvae of fishes and crustaceans may have greater survival chances, if they can adjust their body temperature synchronously with temperature changes of the environmental surface waters. The rate of change in internal body temperature depends upon the specific heat (thermal capacity expressed as cal/unit mass of the organism). Experiments by SHINOZAKI (1957) show that there exists a good correlation between specific heat of grasshoppers and their body water content. Female and male grasshoppers of the genus *Aractomorpha* have specific heat values of 0.74 and 0.68 cal/g; their body water content is 66 and 63%, respectively. Thus, the higher the body water content, the greater the specific heat; consequently, the female grasshopper is able to change its body temperature quicker than the male, and hence may have a greater survival chance. It is conceivable that, having about 90% body water content, planktonic eggs and larvae will be able to adjust their body temperature almost at the same rate as the surrounding surface waters.

Composition and changes in egg membrane weight

The mean dry weight of a single egg membrane of a freshly laid and attached lobster egg (stage I) is 38 µg (2.2% of the egg weight); it increases to 68 µg (4.3% egg weight) in stage III and to 81 µg (5.3% egg weight) in egg stage IV. Such increasing trend indicates that another membrane is added or at least some substances are incorporated into the already existing egg membrane(s) after the egg is attached to the setum. YONGE (1937, 1946, 1955) considered that the crustacean egg basically consists of 2 external non-protoplasmic membranes, in addition to the living membrane situated inside them. The inner non-protoplasmic membrane is of chitin, whereas the outer is formed of epicuticular substances, analogous to the integument of the Crustacea. In decapods such as *Homarus gammarus*, the sequence of events of egg membrane formation and egg attachment is synchronized with the higher activity of the cement gland and, when the egg is finally glued to the setum, it already has both inner and outer egg membranes.

The results obtained in the present study differ from the classical concept of YONGE (1937) (integumental theory), and support the observation of CHEUNG (1966), who investigated the development of egg membranes and egg attachment in the shore crab *Carcinus maenas* and some related decapods in YONGE's laboratory. According to CHEUNG, the outer egg membrane (corresponding to that of YONGE's) really consists of 3 layers, histochemically distinct from one another and not to be equated with epicutile as YONGE (1937) has considered; the outermost layer (layer 1) of this membrane is derived from the vitalline membrane, the second (layer 2), is formed through solidification of a fluid exuded from the egg at spawning, and the third layer (layer 3) is formed by a highly PAS-positive substance from the egg. CHEUNG suggested that the formation of this "trichromatic membrane" is initiated by fertilization. Prior to the appearance of eye-spots (i.e. after the egg is attached to the setum; the period between egg stages I and II of the present study; see p. 155), one (e.g. *Carcinus maenas*) or two (e.g. *Homarus gammarus*) chitinous membranes are formed inside the trichromatic membrane (layers 4 and 5). CHEUNG demonstrated this by subjecting newly attached *Carcinus maenas* and *Astacus astacus* eggs (at an early developmental state) and *Carcinus maenas* and *Homarus gammarus* eggs with eye-spots (at a later developmental stage) to a chitosan test (RICHARDS, 1951). He found that the trichromatic membrane had been dissolved by the KOH in both cases, but in the latter one, something remained behind as boundary membrane. In eggs of *Homarus gammarus* in which 2 such membranes were left behind, they turned brown on application of 0.2% iodine, which is suggestive of the presence of chitin. I have performed the chitosan test on newly attached *Homarus americanus* eggs (at that time the corresponding stage of *Homarus gammarus* was not available) and found that the trichromatic membrane was dissolved completely by KOH (PANDIAN, 1970c), very much similar to the case observed by CHEUNG (1966) in freshly attached eggs of *Carcinus maenas* and *Astacus astacus*. It is very likely that the increase in membrane dry weight from 38 µg in the freshly laid and attached egg (stage I) of *Homarus gammarus* to 68 µg in stage III with eye-spots, is due to the formation of the inner chitinous egg membrane (layers 4 and 5) after the egg is laid and attached to the setum.

To the best of my knowledge, there exist no previous data on chemical composition of the crustacean egg membrane, except for some scattered information which is based on histochemical tests. A single egg membrane (stage IV) of the European lobster weighs 81 µg, or 5.3% of the egg weight; it contains 70.4% protein, 0.13% non-protein nitrogen, 2.8% ash, and contains 4049 cal/g dry weight. VON HENTIG (personal communication) obtained the following values for the brine shrimp *Artemia salina*: egg shell weight = 0.69 µg or 19.7% of egg weight, protein = 77.0%, non-protein nitrogen = 0.32%, fat = 1.9%, carbohydrate = 17.0%, ash = 2.8%, and energy content =

5215 cal/g dry weight. The differences in chemical composition between *Artemia salina* and *Homarus gammarus* eggs, especially egg shell weight, may be related to the peculiarity of the *A. salina* egg, which must be exposed to terrestrial conditions in order to hatch; it has a highly resistant, thick, egg shell. From these data, it is evident that protein represents the major constituent of the egg membrane.

CHEUNG (1966) has drawn the following conclusions from his histochemical and enzyme-digestion studies on the egg membrane of *Carcinus maenas*: (1) The chitosan test indicates the chitinous nature of the inner egg membrane, i.e. layer 4 (and 5 in *Homarus gammarus*); (2) layers 1 and 3 of the trichromatic membrane are both PAS-Sudan Black B positive, suggesting the possibility of their neutral mucopolysaccharide nature; (3) enzyme (amylase, pepsin, trypsin and chitinase) digestion studies indicate that there is little or no glycogen in the egg membranes, and that the trichromatic membranes are largely non-protein substances. Further studies are needed on this aspect, before any definite conclusion can be reached. However, it may be said that, while explaining similar contradictory data on biochemical composition of the chorion (egg membrane) of the trout, HAYES (1942) reported that the chorion appears to be pseudokeratin, and its dissolution by the hatching enzyme cannot be considered a true hydrolysis, because digestion results in such a small yield of free amino acid.

Egg membrane permeability to water and salt

Since the chitinous inner egg membrane is freely permeable (YONGE, 1946), it is conceivable that the outer trichromatic egg membrane is the membrane which controls (a) changes in permeability to water, and (b) selective permeability to salts. In the developing eggs of *Homarus gammarus*, it is shown that high initial permeability (during this period nearly 6% of the total water required is absorbed) is followed by a period (stages I to III) during which the egg membrane is almost water impermeable; it then becomes again highly permeable at later stages (from stage III on), when it imbibes water amounting to about 85% of the total requirement. A similar phenomenon has been observed in eggs of teleost fishes; for instance, trout eggs take up about 18% of their water immediately after being laid (NEEDHAM, 1950, p. 38) and then drastically reduce their permeability until hatching (see also KINNE, 1962).

What is the cause for the sudden change in egg permeability to water (after egg stage III)? The following possibilities have been suggested: (1) Either the accumulation of excretory substances and/or secretion of substances of osmotic value by special glands or gland cells in the developing embryo at that particular stage causes the sudden change in permeability (MARSHALL and ORR, 1954); (2) a special hatching enzyme whose function is the chemical alteration of the egg membrane (DAVIS, 1959); (3) denaturation of the astaxanthin-protein complex seems to have a bearing on the mechanism through which the egg membrane is made permeable again, as the release of free astaxanthin (turning the egg completely red) marks the beginning of the permeability of the egg membrane and many other important changes (see also p. 156). Variations in the egg's osmoconcentration would, of course, decisively affect the net transport of water.

Hatching mechanism

Hatching is effected by a number of means in crustaceans; these may first be summarized:

(1) Osmotic hatching: The developing egg imbibes water through the egg membrane; as it swells, the membrane breaks in due time. This simple and economic mechanism is the only means of egg hatching in cladocerans (RAMULT, 1930) and copepods (MARSHALL and ORR, 1954; DAVIS, 1959).

(2) Osmotic hatching aided by mechanical forces: In addition to the osmotic hatching described, the process of bursting the egg membrane is accelerated by movements of the embryo. Muscular movement has been reported, for example, in *Artemia salina* (NEEDHAM and NEEDHAM, 1930), operation of caudal pressure, e.g. in *Hemimysis* (NEEDHAM, 1931, p. 1601), active extension of the pleon, e.g. in *Palaemonetes vulgaris* (BURKENROAD, 1947) and biting the egg membrane with "egg teeth", e.g. in *Gammarus* species (LE ROUX, 1933).

(3) Osmotic hatching (?) aided by the female: Extract of the "shell" (tissues within, and hypodermis lining of the shell, together with attached muscle ends) of the mother animals has been shown to stimulate mass hatching of barnacles and, in general, in all cirripedes (CRISP, 1956, 1969; CRISP and SPENCER, 1958). This hatching substance represents a product of barnacle tissue metabolism, and is a relatively stable, easily diffusible molecule, which forms lactone at low pH. The hatching substance activates hatching not by altering the egg (membrane) case, but rather by stimulating the movements of the embryos. From the point of mode of action, it is not analogous to the hatching enzyme of fish, which actually digest the protein moiety of the egg membrane (chorion) and thus, effect hatching (SMITH, 1957).

DAVIS (1964a), who described the events of eclosion in the lobster *Homarus americanus*, regarded the mechanism involved as simple osmotic hatching. While I cannot yet pinpoint which of the mechanism or mechanisms mentioned above are involved in the hatching of lobster eggs, the evidence accumulated in the present study does not permit me to accept the view of DAVIS as such. The following points should be considered:

(a) According to DAVIS (1964a), imbibition of water leading to internal pressure results in bursting of the egg membrane and thus, hatching is initiated. Water content of different samples of eggs collected from either the same mother animal or from different mother animals did not vary appreciably from that of other samples of corresponding stages throughout the course of embryonic development (Table 1). Water content of the larvae collected on different (1st, 2nd, 3rd, etc.) days of hatching was remarkably constant (83.1 ± 1.1%). This being so, why did only about 1500 eggs hatch on the first day and only around 10 p.m., and the rest (at the rate of about 1500 eggs/day) for a period of 7 to 10 days? Why did not all the eggs, which had the same water content and hence the same internal pressure, hatch out on the same day?

(b) Let us assume that, after egg stage IV, there are, for some reason, differences in the rate of water absorption among eggs, and only embryos which reach a pressure equivalent to 83.1% water content hatch, and that this fact explains why hatched larvae have a minimum content of 83% water. Inflow of salts increases the osmotic pressure, since "osmotic concentration is dependent on the total number of solute particles independent of size or chemical nature of the dissolved material... the higher the concentration of solute, the higher is the osmotic pressure" (PROSSER and BROWN, 1962, p. 6). *Homarus gammarus* eggs absorb salts faster than water (Fig. 1). It is very likely that embryos (stage IV) continue to absorb salts, even after hatching. In fact, it has been observed that the salt (ash) content of larvae hatched on the first day, was only 16.7%, while that of those hatched on the second and fourth day was 19.3 and 26.7%, respectively (PANDIAN, 1970a). Such a continued uptake of salts must increase the inside osmotic pressure; why is it, that the larvae do not hatch earlier?

(c) The answer seems to be this: Dry weight of the egg membrane increases from 39 µg in stage I to 81 µg in stage IV; as a result, thickness and elasticity of the egg membrane appear to increase so much that the egg can expand to nearly 2 or 3 times its initial size.

(d) If the increase in pressure causes the bursting of the egg membrane, such bursting can occur anywhere, as equal force is applied in all directions. However, cracks invariably occur in the cephalic region. DAVIS (1959, 1964a, b) also observed bursting always to occur in the cephalic region in the shrimp *Potimirim glabra* and the lobster *Homarus americanus*. Only the outer trichromatic membrane bursts; hence, bursting cannot be caused by cephalic or caudal spines, as these would also pierce the inner chitinous membrane. This suggests that, in the cephalic region, a weakened line in the outer trichromatic membrane is formed. How does this happen?

(e) DAVIS (1964a) could also not explain what stimulates the mother lobster to rapidly move its swimmerets in a characteristic pattern during larval hatching.

It may be suggested that the clock, which sets the hatching time, is within the egg itself; however, the time is not only determined by the increase in internal pressure resulting from water inflow, but also by some unknown factor.

Cleidoic properties of marine demersal eggs

The primary energy source for embryonic metabolism of *Homarus gammarus* is fat oxidation; of the 4.20 cal expended on metabolism, as much as 87.7% energy is drawn from fat oxidation. The available data on yolk constituents oxidized as energy sources for embryonic metabolism are summarized in Table 11; marine demersal eggs draw from about 60 to 88% energy from fat oxidation. Two low values (44 and 55%) given here for barnacles, were calculated from

Table 11. *Yolk constituents oxidized as energy source for embryonic metabolism in demersal eggs of some marine animals*

Species	Carbohydrate (%)	Protein (%)	Fat (%)	Reference
Squid *Sepia officinalis*	—	51.5	—	NEEDHAM (1950)
Squid *Loligo vulgaris*	1.0	37.5	61.5	STOLFI (1933)
Limpet *Crepidula fornicata*	6.3	18.8	65.3	PANDIAN (1969)
Barnacle *Balanus balanoides*	38.9	16.7	44.4	BARNES (1965)
Barnacle *Balanus balanus*	26.6	18.7	54.7	BARNES (1965)
Hermit crab *Eupagurus* sp.	—	28.4	66.6	PANDIAN and SCHUMANN (1967)
Shrimp *Crangon crangon*	—	20.8	75.0	PANDIAN (1967)
Lobster *Homarus gammarus*	2.3	13.3	87.7	PANDIAN (this paper)
Isopod *Ligia oceanica*	3.0	9.1	87.9	PANDIAN (1970b)
Average	13.0	23.9	68.0	

data by BARNES (1965, p. 329). Quantities of yolk substances, reported to have been oxidized during the embryonic development of the barnacles, were obtained by subtracting the data pertaining to eggs prior to hatching (comparable to egg stage IV in the present study) from those of fresh eggs. It is after stage IV, that the embryo relies heavily upon energy released by fat oxidation. Had BARNES analyzed the larvae too, he might also have presented values comparable to the others given in Table 11. Upon oxidation, fat releases larger quantities of metabolic water (1 g fat: 1.07 g water; 1 g carbohydrate: 0.56 g water; 1 g protein: only 0.41 g water); unlike protein, oxidation of fat and carbohydrate does not result in ammonia production, the removal of which requires considerable amounts of water. These 2 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 to fresh-water inhabitants.

NEEDHAM (1950) has attributed the following 3 metabolic properties to terrestrial cleidoic eggs: (1) Independence from the environment of water and salts; (2) suppression of protein metabolism; (3) "gearing up" of fat metabolism. There are a few exceptions to NEEDHAM's concept; some terrestrial cleidoic eggs, such as those of insects, depend upon their environment for water; the aquatic, non-cleidoic eggs of fresh-water animals do not depend upon the environment for salts. The non-cleidoic, marine demersal eggs, while heavily dependent on the surrounding sea water for water and salts, display considerable suppression of protein metabolism, and remarkable enhancement of fat metabolism. Non-cleidoic eggs of fresh-water animals utilize 66.1 and 27.1% energy by oxidation of protein and fat, respectively, while cleidoic, terrestrial eggs use 80.7% fat energy and 6.9% protein energy (PANDIAN, 1970d). The marine demersal eggs draw as much as 68% energy from fat oxidation and only 24% energy from protein oxidation (Table 11). In other words, suppression of protein metabolism is about a tenth in terrestrial eggs and one third in marine demersal eggs, as compared to fresh-water eggs. Therefore, as regards these two metabolic properties — suppression of protein metabolism and "gearing up" of fat metabolism — marine demersal eggs are more similar to terrestrial cleidoic eggs, than to other aquatic non-cleidoic eggs of fresh-water animals.

Summary

1. Changes in chemical composition and calorific content, as well as yolk-utilization efficiency have been studied in the developing eggs and embryos of the European lobster *Homarus gammarus* LINNAEUS. Special attention has been given to water metabolism, chemical composition and changes in weight and permeability of the egg membrane, and the hatching mechanism.

2. During embryonic development, some yolk constituents increase, e.g. water from 54.0 to 83.1%, ash from 2.7 to 16.7%, protein from 47.4 to 50.9%, and non-protein nitrogen from 1.0 to 2.4%. At the same time, fat content steadily decreases from 43.8 to 25.4%; this decrease is reflected in depletion of energy content of the eggs from 6343 to 5431 cal/g dry organic substance.

3. Cumulative yolk utilization efficiency during total development is 81.8% for dry weight, 70.0% for organic substance, 60.1% for energy, 87.7% for protein, 47.4% for fat, and 73.2% for carbohydrate.

4. A single egg contains 10.49 calories; of the 4.20 calories expended on metabolic processes of the embryo, only 13.3% energy is drawn from protein oxidation, while fat oxidation supplies as much as 87.7% energy, and carbohydrate oxidation only 2.3%.

5. Progression of embryonic development not only increases the body weight of the embryo, but also the energy content per unit weight of its body (from 4712 to about 5400 cal/g dry weight). During embryonic development a remarkable decrease in net yolk utilization efficiency occurs; it falls from 85.5% in early developmental stages, to 69.4% in later stages.

6. The mean dry weight of a single egg membrane increases from 38 µg (2.2% of the egg weight) in a freshly laid and attached egg, to 68 µg (4.3% of the egg weight) in an egg with eye-spots, and to 81 µg (5.3% of the egg weight) in an egg approaching hatching. This finding supports the observation of CHEUNG (1966) that the formation of the inner chitinous egg membrane occurs after the egg is laid and attached to the setum; possibly more substances are added to increase the thickness of the egg membrane. The egg membrane has the following composition: 70.4% protein, 0.13% non-protein nitrogen, 2.83% ash; its energy content is 4049 cal/g dry weight.

7. After being laid and attached to the setum, the egg membrane is permeable to water and imbibes 6% of the total water required. This initial high permeability is followed by a period during which the egg membrane is almost impermeable to water; at a later developmental stage the egg membrane imbibes 85% of the total amount of water required (the rest, 9% is supplied by metabolic water). The egg membrane is selectively permeable to salts throughout development; the rate of salt intake almost doubles in eggs approaching hatching.

8. A simple osmotic hatching mechanism, as proposed by previous workers, is shown to be inadequate to explain the events and timing of the hatching process in the European lobster. It is suggested that the mechanism which determines the hatching time lies within the egg itself; hatching time is determined not only by increased internal pressure resulting from

inflowing water, but also by some unknown endogenous factor.

9. A single egg (3.69 mg wet weight) requires as much as 4.88 mg water for the successful completion of embryonic development. It is suggested that accumulation of water in developing marine demersal eggs offers the following advantages to egg and/or larva: (a) it serves in osmotic hatching of the egg, (b) it floats the hatched larva by means of specific gravity reduction, (c) it aids the larva to adjust its body temperature quickly.

10. In eggs of *Homarus gammarus*, as well as in many other marine demersal eggs, protein metabolism is suppressed to a considerable extent, and fat metabolism "geared up". Thus, these non-cleidoic eggs exhibit metabolic properties, which are typical of cleidoic eggs. This observation is discussed in the light of NEEDHAM's (1950) concept of "cleidoic-terrestrial and non cleidoic-aquatic eggs".

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