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Yolk utilization in the gastropod *Crepidula fornicata**

T. J. PANDIAN

Biologische Anstalt Helgoland (Meeresstation); Helgoland, Germany (FRG)
and
Zoology Department, Bangalore University; Bangalore, India

Abstract

The increases in constituents per unit weight of eggs during embryonic development of the gastropod *Crepidula fornicata* amounted to 14.6% for ash, 1.0% for protein, and 0.3% for non-protein nitrogen. During the same stages, fat content decreased from 33.7 to 20.3%, carbohydrate from 10.2 to 7.7% and energy content from 6209 to 5298 cal/g dry organic substance. The cumulative efficiencies for yolk utilization were 83.8% for dry weight, 61.0% for energy, 85.1% for protein, 50.7% for fat, and 63.6% for carbohydrate. A single egg contained 0.0269 cal, a single veliger 0.0164 cal. Of the 0.0105 cal expended on metabolic processes of the embryo, oxidation of fat contributed as much as 65.3%, while that of protein and carbohydrate amounted only to 18.8 and 6.3%, respectively. On the basis of ecophysiological considerations, a new classification of eggs is proposed.

Introduction

This paper is based on earlier studies dealing with quantitative aspects of matter and energy transformations from yolk to embryo of aquatic thermo-conformers, especially the sole *Solea solea* (FLÜCHTER and PANDIAN, 1968), the shrimp *Crangon crangon* (PANDIAN, 1967 a, b), and the hermit crab *Eupagurus bernhardus* (PANDIAN and SCHUMANN, 1967). It has been shown that freshly-hatched larvae of shrimp and hermit crab utilized about 60% of the energy contained in the eggs and that oxidation of fat was the main energy source for metabolism of the embryo. The present paper reports on changes in chemical composition and calorific content of developing eggs of the slipper limpet *Crepidula fornicata*.

Material and methods

Material: As in the oyster, the first settled limpet forms the substratum for settlement of subsequent specimens; thus, there may be about 10 individuals attached to each other. The slipper limpet is a protandric hermaphrodite; the male takes about 60 days for the completion of sex transformation. Spawning commences in May and continues till early September; the eggs are laid in a bunch of stalked, balloon-shaped capsules, united to a common stem, fastened to the substratum and protected by the parent shell whilst development is in progress. For further details on the bionomics of *Crepidula fornicata* consult CHIPPERFIELD (1951). Our slipper limpets were collected near Helgoland (Southern North Sea) during August-September, 1967. They were kept in large aquaria (capacity 1500 l) containing running seawater (31‰ S) maintained at an ambient temperature of 14 °C.

For chemical analyses, the following arbitrary stages were chosen:

Stage I: Undeveloped eggs and cleavage stages, the earliest stages obtainable; the eggs are round-to-oval in shape, with an average diameter of about 160 µ, and deep yellow-to-scarlet in color.

Stage II: "Pre-veliger" stage in which gastrulation is complete and the archenteron and stomodaeum are well formed; the shell gland can usually be seen. The light yellow-colored embryo is well ciliated.

Stage III: "Late-veliger" stage, ready to hatch. The shell is well formed and the velum 4-lobed and very strongly ciliated; the margin of the velum is also darkly pigmented. The foot is well developed, ciliated, and darkly pigmented. The embryo is brown or dark grey.

Stage IV: Freshly hatched veligers, about 250 µ long and 175 µ broad.

Methods: Of each of the above mentioned stages 3,000 to 4,000 eggs or veligers were counted. As seawater adhering to the egg surface may increase ash weight by about 20% (FLÜCHTER and PANDIAN, 1968), the eggs and veligers were washed free from the adhering seawater by exposing them to distilled water; when exposed to distilled water for periods varying from 1 to 3 min, they secreted large quantities of a jelly-like slime substance. Slime substance increases the dry weight and ash content and decreases the calorific content of the test material (FLÜCHTER and PANDIAN, 1968). Secretion of slime by developing eggs of marine animals may be considered as a mechanism for temporarily escaping unfavorable conditions. In the present study, it was possible to reduce the slime secretion by exposing the test material to distilled water for a period of 15 to 25 sec only. The test material was then dried at 80 °C for 4 h and weighed in a Sartorius balance (type 2604; sensitive to ± 10 µg).

Ash content was estimated by keeping the sample in a crucible of known weight in a muffle furnace at 550 °C for a period of 5 h 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 non-protein nitrogen, while the precipitate was protein (GIESE et al., 1959). Protein content was determined estimating nitrogen

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in a micro-Kjeldhal unit (ROTH, 1958) and multiplying the values obtained by 6.25.

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

Calorific content was determined with a PARR 1412 semi-micro bomb calorimeter. Since the samples available were less than 15 mg in most cases, a known amount of benzoic acid was added as trigger substance.

Results

Changes in chemical composition

As many as 10 egg capsules containing stage I were carefully counted; on an average each egg capsule contained 244 eggs. This number compares favorably with that (about 250 eggs/capsule) reported by CHIPPERFIELD (1951) for the slipper limpet from the river Blackwater, Essex, and that (about 250 eggs/capsule) reported by THORSON (1946) for the slipper limpet from Danish waters. Taking the average value of 244 eggs per capsule, about 4000 eggs were collected for each estimate by counting the egg capsules. This was, however, possible only in egg stages I, II, and III; the veligers (stage IV) had to be counted individually.

Table 1. *Dry weight estimations of different developmental stages of the slipper limpet Crepidula fornicata*

Developmental stage	Number of estimates	Number of eggs	Mean dry weight per egg (µg)	Coefficient of variation
Stage I (egg)	7	28000	4.38 ± 0.25	5.7%
Stage II	4	16000	4.14 ± 0.17	4.1%
Stage III	4	16000	4.12 ± 0.24	5.8%
Stage IV (veliger)	5	15000	3.67 ± 0.27	7.4%

Table 1 presents the total number of estimates and eggs of each stage used to determine the average dry weight of a single egg and the changes in its dry weight during the ensuing developmental stages. The mean dry weight of an egg decreases from 4.38 µg in stage I to 4.12 µg in stage III. The mean dry weight of a single veliger is 3.67 µg; thus, the total loss amounts to 0.71 µg during the entire embryonic development. Although weight and body size ranges of egg-bearing females were restricted (about 2 g; 3.7 cm long × 2.5 cm shell width), egg size deviates up to about 5.0% from the mean value, that of the larva by as much as 7.4%. Such variations in egg and larva size appear to be not uncommon among other marine animals; they have, for example, been reported in cirripedes by BARNES and BARNES (1965), and in shrimp and European and Canadian lobsters by PANDIAN (1967 a, b; 1969 a, b).

Changes in chemical composition of the 4 developmental stages are reported in Table 2. Ash content, which was 1.3% in stage I, increases to 15.9% in stage IV. Stage III (i.e. embryo just prior to hatching), has 20.6% ash content; it is not clear whether the embryo (stage IV) lost some ash substance before hatching or whether the duration of exposure to distilled water (20 sec) was not long enough for stage III.

Table 2. *Changes in chemical composition of developing eggs and freshly hatched veligers of Crepidula fornicata. Percentage values represent means of 3 or 4 estimates and are based on dry weight*

Developmental stage	Ash (%)	Protein (%)	Non-protein nitrogen (%)	Fat (%)	Carbohydrate (%)
Stage I (egg)	1.3	53.6	1.2	33.7	10.2
Stage II	4.8	53.0	1.4	—	—
Stage III	20.6	53.8	1.4	—	—
Stage IV (veliger)	15.9	54.6	1.5	20.3	7.7

Table 3. *Changes in calorific content of developing eggs and freshly hatched veligers of Crepidula fornicata. Calorific values represent means of 3 to 4 estimates*

Developmental stage	Energy content (cal/g dry weight)	Coefficient of variation (%)	Energy content (cal/g dry organic substance)
Stage I (egg)	6128 ± 272	4.4	6209
Stage II	5949 ± 177	3.0	6247
Stage III	4196 ± 195	4.6	5284
Stage IV (veliger)	4456 ± 513	11.8	5298

Protein content of developing eggs remains relatively unchanged; it increases from 53.6% in stage I to 54.6% in stage IV. During these stages, non-protein nitrogen increases from 1.2 to 1.5%.

Fat content shows a remarkable decrease from 33.7% in stage I to 20.3% in stage IV, amounting to a heavy loss of 33.8% of the initial value.

No direct estimation of carbohydrate has been made. Since dry weight, ash, protein, non-protein nitrogen and fat contents are known, carbohydrate content was calculated. Carbohydrate decreases from 10.2% in the fresh egg to 7.7% in stage IV.

Table 3 reports the changes in calorific content of eggs during the developmental stages. Calorific content decreases from 6209 cal/g in the fresh egg to 5298 cal/g dry organic substance in the veliger.

Efficiency of yolk utilization

From the values presented in Tables 1 to 3, average changes in chemical composition and calorific content of a single egg from stage I to stage IV have

been calculated. The values obtained are shown in Table 4. Ash and non-protein nitrogen contents increase, while all other constituents of the egg decrease, as development progresses. Cumulative efficiency values (ratio "body formed/body formed + yolk used for metabolism") have been calculated. The efficiency values are 83.8% for dry weight, 71.5% for organic substance, 61.0% for energy, 85.1% for protein, 63.6% for carbohydrate, and 50.7% for fat. The difference in the values indicates that the efficiency with which different substances of the yolk are utilized, varies considerably.

During the embryonic development, as much as 0.0105 cal were used for metabolic processes (Table 4). Of this amount, protein (0.35 µg protein used × 5650 cal/g protein = 0.00198 cal) and carbohydrate (0.16 µg carbohydrate used × 4150 cal/g carbohydrate = 0.00066 cal) contribute 18.8% and 6.3%, respectively, while fat oxidation (0.73 µg fat used × 9400 cal/g fat = 0.00686 cal) supplies as much as 65.3% energy. Thus, fat oxidation is the main energy source for the embryonic metabolism of the slipper limpet.

Table 4. *Average changes in chemical composition and calorific content in a developing egg and a freshly-hatched veliger of Crepidula fornicata. All weight units are given in µg. (Data based on Tables 1 to 3)*

Parameter	Stage I (egg)	Stage II	Stage III	Stage IV (veliger)
Dry weight	4.38	4.14	4.12	3.67
Ash	0.06	0.20	0.85	0.58
Organic substance	4.32	3.94	3.27	3.09
Protein	2.35	2.20	2.22	2.00
Non-protein nitrogen	0.05	0.06	0.06	0.06
Fat	1.48	—	—	0.75
Carbohydrate	0.44	—	—	0.28
Energy (cal/egg)	0.0269	0.0246	0.0173	0.0164

Discussion

The changes in relative proportions of yolk constituents during the embryonic development of the slipper limpet *Crepidula fornicata* are similar to those reported for the shrimp *Crangon crangon* (PANDIAN, 1967 b) and the hermit crab *Eupagurus bernhardus* (PANDIAN and SCHUMANN, 1967). Ash content increases from 1.3% in the fresh egg to 15.9% in the veliger; each egg absorbs 0.52 µg (ash) salt during embryonic development. It is well known that marine eggs absorb salt from the surrounding seawater. For instance, it has been shown that the egg of the squid *Sepia* contains 0.8 mg of ash at the beginning of development and no less than 3.3 mg at the end (BALDWIN, 1964, p. 12). While the protein content of developing slipper limpet eggs remains relatively unchanged, the fat content shows a remarkable decrease from 33.7% in the fresh egg to 20.3% in the veliger; this is also reflected in calorific content, which decreases from 6209 cal/g to 5298 cal/g dry organic substance, in the corresponding stages. Thus, ash content on the whole increases 14.6%, while fat content decreases 13.4%. Therefore, fat and ash are the 2 important variables during embryonic development of the slipper limpet.

The efficiency values obtained for the slipper limpet compare well with those obtained for crustaceans (PANDIAN, 1967 b; PANDIAN and SCHUMANN, 1967) and fish (LASKER, 1962; FAUSTOV and ZOTIN, 1964). The primary energy source for the embryonic metabolism of the slipper limpet is fat oxidation. Of the 0.0105 cal expended on metabolism, as much as 65.3% is supplied from fat oxidation, while that of protein and carbohydrate amounts to only 18.8% and 6.3%, respectively. STOLFI (1933), using the squid *Loligo vulgaris*, found that 61.5% of the total energy used for embryonic metabolism is drawn from fat oxidation. According to OCKELMANN (personal communication), the dense fat globules observed in fresh eggs of many marine lamellibranchs disappear as development proceeds, suggesting the utilization of considerable quantities of fat. During the development of many marine crustaceans, fat serves as the main energy source, e.g. in *Crangon crangon*, *Homarus gammarus*, *H. americanus*, *Ligia oceanica* (PANDIAN, 1967 b, 1969 a, b, c), and *Eupagurus bernhardus* (PANDIAN and SCHUMANN, 1967). Upon oxidation, fat releases large quantities of water (1 g fat 1.07 g water; 1 g protein 0.41 g water; 1 g carbohydrate 0.56 g water; BALDWIN, 1964, p. 52); unlike protein, fat oxidation does not result in ammonia production, the removal of which costs considerable quantities 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 freshwater inhabitants.

Such a generalization differs from the concept of NEEDHAM (1950, pp 49 to 51). Depending on the nature of the substance used as energy source during the embryonic development, NEEDHAM distinguished terrestrial eggs, which derive the required energy primarily by oxidizing their fat, from all aquatic eggs with protein as main energy source. Let us look at the data from which NEEDHAM formulated his concept (Table 5). Of the 9 aquatic species, only 4 are marine species; obviously, marine species are not sufficiently represented. Of the 4 marine species chosen, the *Loligo vulgaris* embryo uses as much as 61.5% fat energy for its metabolism, and *Sepia officinalis* and *Paracentrotus lividus* embryos use only about 50% protein energy for their metabolism. Only in the plaice *Pleuronectes platessa* does 90% of the energy for embryonic metabolism come from protein oxidation; the possible reason for this is discussed below. Averaging the values for the poorly-represented marine species and the relatively well-represented freshwater species, NEEDHAM could not reveal the characteristic feature of marine eggs, namely the use of fat as main energy source.

Physiologically, the marine environment shares with the terrestrial environment the restricted availability of water. I therefore propose a classification of eggs based on the environment (marine, freshwater and terrestrial eggs). NEEDHAM's (1950) conception of the different nature of substances used for embryonic metabolism may be taken for sub-classifying marine, freshwater and terrestrial eggs. Considering marine fish eggs alone, the plaice (*Pleuronectes platessa*) embryo uses about 90% of protein energy for its metabolism, and it is likely that the embryos of the Pacific sardine *Sardinops caerulea* (LASKER, 1962) and the sole *Solea solea* (FLÜCHTER and PANDIAN, 1968) also use protein as main energy source. The calorific content of their fresh eggs is about 5800 cal/g dry organic substance and, instead of a decrease, there is a slight increase (6000 cal/g dry organic substance) in the calorific content of freshly-hatched larvae, suggesting that a substance of low calorific content, possibly protein, is used for embryonic metabolism. On the other hand, the calorific contents of the freshly-spawned eggs and freshly-hatched larvae of the herring *Clupea harengus* amount to 6585 cal/g and 5940 cal/g dry organic substance, respectively (PAFFENHÖFER and ROSENTHAL, 1968), indicating that the herring embryo uses mainly fat energy. Thus, there may be 2 types of marine fish eggs: (1) those with fat as main energy source, e.g. the herring *Clupea harengus*, and (2) those with protein as main energy source, e.g. the plaice *Pleuronectes platessa*.

What could be the ecological advantage for embryos of the plaice, the Pacific sardine, and the sole, to oxidize protein as metabolic energy source? The following may be suggested: the eggs of all 3 species are planktonic; egg floatation is brought about through reduction of the egg's specific gravity (for instance, specific gravity of the plaice egg before ripening is 1.070, after ripening 1.025; SMITH, 1957); this may be achieved by altering the protein: fat (yolk) ratio (proportion) of the egg. A shift towards greater deposition of protein instead of fat (yolk) results in imbibition of water. Protein molecules are highly lyophilic, while fat molecules are lyophobic; as much as 3 g water are associated with 1 g protein, but only 0.1 g water with 1 g fat (BRODY, 1945, p. 52). For this reason, planktonic marine eggs contain as much as 90% water (low calorific content, about 600 cal/g wet weight), but demersal marine eggs only 76% (high calorific content about 1650 cal/g wet weight) e.g. herring eggs (FLÜCHTER and PANDIAN, 1968). It seems tempting then, to postulate that protein accumulation in marine planktonic eggs as main energy source for embryonic metabolism is primarily related to the required reduction in specific gravity.

Table 5. *Yolk constituents oxidized as energy source for embryonic metabolism in the eggs of different animals. (Data from* NEEDHAM, *1950; rearranged)*

	Species	Carbohydrate (%)	Protein (%)	Fat (%)
Terrestrial	Chick (*Gallus domesticus*)	3.0	5.6	91.4
	Grasshopper (*Melanopus* sp.)	—	—	72.5
	Silkworm (*Bombyx mori*)	26.0	10.0	64.0
	Sheep blowfly (*Lucilia sericata*)	Trace	5	95
Intermediate: Turtle (*Thalassochelys*)		—	19	81
Aquatic	Frog (*Rana temporaria*)	6.8	70.7	22.4
	Salamander (*Cryptobranchus* sp.)	12.3	51.5	36.4
	Carp (*Cyprinus carpio*)	—	61.5	23.8
	Trout (*Salvelinus fontinalis*)	—	63	37
	Trout (*Salmo irideus*)	Trace	83.8	16.0
	Plaice (*Pleuronectes platessa*)	—	90	—
	Sea-urchin (*Paracentrotus lividus*)	21.0	50.0	29.0
	Squid (*Sepia officinalis*)	—	51.5	—
	Squid (*Loligo vulgaris*)	1.0	37.5	61.5
Averages: Terrestrial			6.8	80.7
Aquatic			62.1	32.3

Summary

1. Changes in chemical composition and calorific content in developing eggs of the gastropod *Crepidula fornicata*, as well as their yolk utilization efficiency, have been studied.

2. During the entire embryonic development, ash content per unit egg weight increases 14.6% (0.52 µg/embryo); there is a more or less parallel decrease of 13.4% in fat content during corresponding developmental stages. This is reflected in changes of calorific content, which decreases from 6209 cal/g to 5298 cal/g organic dry substance. Protein and carbohydrate contents remain relatively unchanged.

3. The cumulative efficiencies of yolk utilization are 83.8% for dry weight, 61.0% for energy, 85.1% for protein, 63.6% for carbohydrate, and 50.7% for fat.

4. The average calorific contents of a single egg and a single veliger are 0.0269 cal and 0.0164 cal, respectively. Of the 0.0105 cal expended on embryonic metabolism, as much as 65.3% is supplied by fat oxidation; protein and carbohydrate supply only 18.8% and 6.3%, respectively.

5. The results are discussed in the light of NEEDHAM's (1950) egg classification and on the basis of ecophysiological considerations.

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Literature cited

BALDWIN, E.: An introduction to comparative biochemistry, 4th ed., 179 pp. Cambridge: University Press 1964.

BARNES, H. and M. BARNES: Egg size, nauplius size, and their variation with local, geographical and specific factors in some cirripedes. J. Anim. Ecol. **34**, 391—402 (1965).

BRODY, S.: Bioenergetics and growth, 1023 pp. New York: Rheinhold 1945.

CHIPPERFIELD, P. N. J.: The breeding of *Crepidula fornicata* in the river Blackwater, Essex. J. mar. biol. Ass. U.K. **30**, 49—71 (1951).

FAUSTOV, V. S. and A. I. ZOTIN: Changes in the heat of combustion of the eggs of fishes and amphibians during development. Dokl. Akad. Nauk SSSR (Biol. Sci.) **162**, 320—323 (1964).

FLÜCHTER, J. and T. J. PANDIAN: Rate and efficiency of yolk utilization in developing eggs of the sole *Solea solea*. Helgoländer wiss. Meeresunters. **18**, 53—60 (1968).

GIESE, A. C., L. GREENFIELD, H. HUANG, A. FARMANFARMIAN, R. BOOLOOTIAN and R. LASKER: Organic productivity in the reproductive cycle of the purple sea-urchin. Biol. Bull. mar. biol. Lab., Woods Hole **116**, 49—58 (1959).

LASKER, R.: Efficiency of yolk utilization by developing embryos and larvae of the Pacific sardine *Sardinops caerulea* (GIARD). J. Fish. Res. Bd Can. **19**, 867—875 (1962).

NEEDHAM, J.: Biochemistry and morphogenesis, 785 pp. Cambridge: University Press 1950.

PAFFENHÖFER, G. A. und H. ROSENTHAL: Trockengewicht und Kaloriengehalt sich entwickelnder Heringseier. Helgoländer wiss. Meeresunters. **18**, 45—52 (1968).

PAINE, R. T.: Ash and calorie determinations of sponge and opisthobranchs tissues. Ecology **45**, 384—387 (1964).

PANDIAN, T. J.: Preliminary report on chemical composition and caloric content of developing eggs of the shrimp *Crangon crangon*. *In:* C. M. (Council Meeting) I.C.E.S. (International Council for the exploration of the sea) Sect. Shellfish Committee K **12**, 1—8 (1967 a).

— Changes in chemical composition and caloric content of developing eggs of the shrimp *Crangon crangon*. Helgoländer wiss. Meeresunters. **16**, 216—224 (1967 b).

— Physiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. (1969 a) (unpublished).

— Yolk utilization and hatching time in the Canadian lobster *Homarus americanus*. (1969 b) (unpublished).

— Changes in chemical composition and calorific content of developing eggs of the ovoviviparous isopod *Ligia oceanica*. (1969 c) (unpublished).

— and K. H. SCHUMANN: Chemical composition and caloric content of egg and zoea of the hermit crab *Eupagurus bernhardus*. Helgoländer wiss. Meeresunters. **16**, 225—230 (1967).

ROTH, H.: Quantitative organische Mikroanalyse, 7. Aufl. 361 pp. Wien: Springer 1958.

SMITH, S.: Early development and hatching. *In:* The physiology of fishes, Vol. 1. pp 323—360. Ed. by M. E. BROWN. New York: Academic Press 1957.

STOLFI, G.: L'accrescimento embrionale del *Loligo vulgaris*. Atti. Accad. naz. Lincei Rc. (Cl. Sci. fis. mat. nat.) **18** (6), 516 (1933).

THORSON, G.: Reproduction and larval development of Danish marine bottom invertebrates. Meddr Kommn Havunders. (Ser. Plankton) **4**, 1—523 (1946).

Author's address: Dr. T. J. PANDIAN
Zoology Department
Bangalore University
Bangalore 1, India

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