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CHANGES IN COMPOSITION, WEIGHT AND ENERGY CONTENT DURING THE LECITHOTROPHIC DEVELOPMENT OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

CAMBIOS DE LA COMPOSICIÓN, PESO Y CONTENIDO ENERGÉTICO DURANTE EL DESARROLLO LECITOTRÓFICO DEL CAMARON BLANCO DEL PACÍFICO *Litopenaeus vannamei*

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ABSTRACT

There is little information on the energy budget of *Litopenaeus vannamei* during its larval development, and none is available for the stages that depend only on their egg reserves. In this work we measured the changes in total and organic individual dry weight, biomass composition and energy content of *L. vannamei* eggs, nauplius I, III and V and protozoea I (E, N_1, N_3, N_5 and PZ_1), obtained in 12 spawns from a commercial hatchery. The average duration of embryonic development was 9 h and the time needed to reach dominance (>70%) of PZ_1 was four days. The mean total dry weight of the eggs was 4,32±0,23 µg and decreased from N_1 through N_5 (3,52±0,28 µg) but increased after metamorphosis into PZ_1 due to a strong increase of the inorganic content. This increased from 10,5±0,1 to 19,6±0,4%, whereas the individual organic weight decreased continuously, from 4,12±0,22 µg (E) to 3,09±0,39 µg (PZ_1). Protein and carbohydrate contents remained unchanged throughout larval development, with respective mean values of 35,2±1,4 and 2,6±0,3%, but the percentage of lipids decreased progressively from 29,1±2,5 to 20,2±2,1%, thus showing a preferential consumption of this substrate. The caloric content decreased from 105,0±6,3 to 55,1±9,4 mJ/ind and the most relevant losses were caused by the costs of embryonic development (17.8 mJ/ind in 9 h) and of the metamorphic change from N_5 to PZ_1 (14.7 mJ/ind in 18 h). These two processes required 65% of the total energy cost of the lecithotrophic development of *L. vannamei*.

Key words: *Litopenaeus vannamei*, lecithotrophic development, weight, composition, energy content.

RESUMEN

Existe poca información sobre el costo energético del desarrollo larvario de *Litopenaeus vannamei* y éste se desconoce para las etapas que dependen solamente de las reservas del huevecillo. En este trabajo se registraron los cambios del peso total y orgánico individual, de la composición de la biomasa y del contenido energético de los huevecillos, de los nauplios I, III y V y de protozoea I (E, N_1, N_3, N_5 y PZ_1) de *L. vannamei* obtenidos en 12 desoves en un laboratorio comercial. La duración media del desarrollo embrionario fue de 9 h y el tiempo necesario para alcanzar la dominancia (>70%) de PZ_1 fue de 4 días. El peso seco total medio de los huevecillos fue 4,32±0,23 µg y disminuyó durante el desarrollo entre N_1 y N_5 (3,52±0,28 µg), pero aumentó después de la metamorfosis a PZ_1 debido a un cambio importante del contenido inorgánico. Éste aumentó desde 10,5±0,1 a 19,6±0,4%, mientras que el peso orgánico individual disminuyó en forma continua de 4,12±0,22 µg (E) a 3,09±0,39 µg (PZ_1). Los porcentajes de proteínas y carbohidratos permanecieron sin cambios durante todo el desarrollo larvario, con valores medios de 35,2±1,4 y 2,6±0,3%, mientras que los lípidos disminuyeron progresivamente desde 29,1±2,5 a 20,2±2,1%, demostrando de esta manera un consumo preferencial de este tipo de reservas. El contenido calórico disminuyó de 105,0±6,3 a 55,1±9,4 mJ/ind y las pérdidas más importantes se registraron durante las 9 h de desarrollo embrionario (17.8 mJ/ind) y las 18 h del cambio metamórfico de N_5 a PZ_1 (14.7 mJ/ind). Estos dos procesos representaron el 65% del costo energético total del desarrollo lecitotrófico de *L. vannamei.*

Palabras clave: *Litopenaeus vannamei*, desarrollo lecitotrófico, peso, composición, contenido energético.

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INTRODUCTION

The ontogenesis-related changes in weight, composition and energy content of crustacean larvae, including lecithotrophic stages, have received considerable attention Anger *et al*. (1989, 1990, 2003); Yagi *et al*. (1990); Anger, (1996); Calcagno *et al*. (2003); Kattner, (2003), but the few studies on penaeid larvae concern mainly their planktotrophic stages.

Some of these give isolated data on the weight or composition of the eggs or nauplii of a limited number of species Kurmaly *et al*. (1989); Chu and Ovsianico-Koulikowsky, (1994); Mourente *et al*. (1995); Palacios *et al*. (1998); Rosas, (1999); Lemos and Phan, (2001), but there is no information on the total cost of their lecithotrophic development, which we provide for this part of the larval development of the Pacific white shrimp *Litopenaeus vannamei*.

MATERIALS AND METHODS

The samples were from twelve spawns (4 in 2002 and 8 in 2003) with farm-raised *L. vannamei* broodstock that were subjected to eyestalk ablation in a commercial hatchery. The initial samples were obtained from the spawning tanks (500 l) immediately after each spawn. Source seawater (temperature 29-30 C; salinity: 34-35 g/l) for the spawning tanks was prepared by filtration to 5-µm, UV-treatment and addition with 1 mg/l Na_2EDTA, to reduce toxicity of heavy metals and also to avoid egg collapse (Mourente *et al*. 1995).

The females were immediately ?????? after spawning, and the eggs remained in the same tank for 7 to 10 h with profuse aeration until hatching of at least 20-25% of the spawn. At this time, aeration was suspended, unhatched eggs sank to the bottom, and the swimming nauplii (N_1) were concentrated using their phototactic response, collected by gentle siphoning and moved to clean tanks with 5-µm filtered, UV-treated seawater. After this, aeration was resumed to allow the remaining eggs to hatch and assess the hatching success.

After hatching, nauplii were evaluated for changes in weight and in composition at the end of embryonic development. Additional samples were obtained once >70% of the larvae had reached the substages N_3, N_5 and PZ_1. The PZ_1 samples were obtained prior to first feeding.

The mean larval mass was determined with samples concentrated on prewashed and preweighed Whatman GF-C glass fiber filters, washed with a 3% ammonium formate solution, dried to constant weight (DW) in an oven at 60 C, and ashed at 450 C to obtain the inorganic content (AW). The resulting values were used to calculate the organic biomass as AFW = DW - AW.

The protein and carbohydrate contents of the larvae were determined with the respective methods by Lowry *et al*. (1951) and (Dubois *et al*. 1956). Lipids were determined as in Pande *et al*. (1963), after extraction following (Bligh; Dyer 1959). The samples for determination of the energy content were frozen at -70 C, freeze-dried, ground in a mortar and used to obtain pellets of known dry weight. The energy content of the pellets was measured with a Parr 1425 semimicro oxygen bomb calorimeter.

All the samples were in triplicate and all contained known numbers of larvae. The average values obtained in each experiment were used to calculate the mean individual DW, AFW, composition and energy content of each larval stage. All data were normal and homoscedastic (Lilliefors' and Bartlett's tests) and were compared by one-way block analysis of variance (ANOVA) with $\alpha = 0.05$. Significantly different mean values were separated with Tukey's tests (Zar, 1996).

RESULTS

On average, the embryonic development until first hatching lasted 9 h, the intervals between naupliar substages were approximately 14 h each, and the change of dominance from >70% N_5 to >70% PZ_1 took 18 h, for a total of 4 d from spawn to the first feeding stage.

The individual DW of the eggs ranged between 4,1 and 4,9 µg, with a mean value of 4,3 µg. After hatching, the mean DW was 3,8 µg, with a relative loss of 12% and the last naupliar stage weighed on average 3,5 µg (81,5% of the value determined for the eggs immediately after spawning). During the metamorphic change into PZ_1 it increased again to 3,8 µg (Table 1).

The mean AFW of the eggs was 4.1 µg/ind (range 3.9-4.7 µg). It decreased by 13% at the end of embryonic development and continued to decrease from N_5 to PZ_1, with a final mean value of 3.1 µg/ind (Table 1).

Table 1. Mean values and standard error of individual total and ash-free weights (DW and AFW, in µg/ind), and energy content (E, in mJ/ind) of *Litopenaeus vannamei* during lecithotrophic development. E = egg; N_1, N_3 and N_5 = nauplius I to V; PZ_1 = unfed protozoea I. N = 12. Equal or common letters indicate lack of significant differences (one way ANOVA, α = 0.05). a≤ab≤b and a<b<c

	E	N_1	N_3	N_5	PZ_1
DW µg	4.32c	3.80b	3.75b	3.52a	3.76b
	(0.07)	(0.11)	(0.10)	(0.06)	(0.07)
AFW µg	4.12c	3.58b	3.43ab	3.15a	3.09a
	(0.07)	(0.10)	(0.09)	(0.07)	(0.11)
E µJ	105.03d	87.24c	78.44c	69.75b	55.05a
	(1.82)	(4.06)	(3.41)	(1.49)	(2.71)

The energy cost of embryonic development was 17.8 mJ/ind, equivalent to 17% of the egg reserves. From N_1 to N_5 the loss was 17.5 mJ/ind and that of metamorphosis from N_5 to PZ_1 was 14.7 mJ/ind, that represent 21% of the content of the previous stage (Table 1).

The rate of energy consumption was 1.98 mJ/h/ind from spawning to hatching. The five naupliar stages lasted approximately 70 h, with a net loss of 17.5 mJ (0.25 mJ/h/ind). During the metamorphosis from N_5 to PZ_1 the rate of energy consumption was 0.82 mJ/h/ind.

The mean weight losses were 16.5 and 25% of the initial DW and AFW, but the energy used was equivalent to 47.5% of the initial reserves. According to the results obtained with these observations, the changes in individual DW, AFW and energy content (E) may be described by the equations:

$$DW = 4.071 - 0.151\ t\ (r^2 = 0.290);$$

$$AFW = 3.92 - 0.253\ t\ (r^2 = 0.634);$$

$$E = 98.16 - 10.27\ t\ (r^2 = 0.694)$$

where DW and AFW are in µg/ind; E is in mJ/ind and t is the age of the larvae in days or fractions of day. In all cases the exponential models gave similar fits, with r^2 values of 0.284 and 0.623 for DW and AFW, and 0.687 for E.

The poor fit of the equations describing the progressive changes of DW may be due to the tendency of the inorganic content, that increased in irregular steps (30, 45 and 25% of the previous value) from the initial 4.45% of the eggs to 10.5% for the N_5 substages, but rose to almost twice this value (19.6%) after the change to PZ_1.

Protein and carbohydrate contents remained virtually unchanged (one way ANOVA, $p > 0.05$), with mean values of 35.2 and 2.54% of the organic biomass (respective SEMs = 0.69 and 0.09), whereas lipids declined progressively from 29.1 to 20.2% (Table 2).

Table 2. Mean and standard error of the ash (AW in % of total weight) and of protein, carbohydrate and lipid content (in % of ash-free dry weight) of *Litopenaeus vannamei* during lecithotrophic development. E = egg; N_1, N_3 and N_5= nauplius I to V; PZ_1 = unfed protozoea I. N = 12. Equal or common letters indicate lack of significant differences (one way ANOVA, α = 0.05). a≤ab≤b and a<b<c<d.

	E	N_1	N_3	N_5	PZ_1
AW %	4.45a	5.80ab	8.43b	10.49c	19.63d
	(0.01)	(0.03)	(0.11)	(0.04)	(0.13)
Protein	33.54a	37.08a	36.65a	34.40a	34.37a
	(0.85)	(1.67)	(1.48)	(1.18)	(1.81)
Carbo-hydrate	2.40a	2.64a	2.62a	2.26a	2.76a
	(0.39)	(0.17)	(0.35)	(0.18)	(0.17)
Lipid	29.10c	25.26b	25.01b	22.43ab	20.15a
	(0.72)	(0.86)	(0.60)	(0.74)	(0.61)

This decline had an almost linear trend, that explains the low value of the determination coefficient obtained with the exponential model ($r^2 = 0.478$). Thus, the equation that describes the progressive decrease of the lipid content of the lecithotrophic stages of *L. vannamei* with the best fit is:

$$Lipids\% = 27.70 - 1.78\ t\ (r^2 = 0.771)$$

DISCUSSION

The number of eggs (120-150,000/female), the percentages of hatching after 14-15 h (60-80%) and of malformed larvae (<10%) were within the normal values obtained in commercial hatcheries before the onset of reproductive exhaustion (Palacios *et al.* 1999). For this reason, we assume that our results are representative of the changes in weight, composition and energy content during the lecithotrophic development of *L. vannamei*, at least under the relatively stable conditions of a commercial hatchery.

Simoes *et al.* (2001) observed the opening of the anal pore of *L. vannamei* N_5 larvae several hours before they moulted to PZ_1, and suggested that this and antiperistalsis of the hindgut (anal drinking) provide a potential route for bacteria colonization before the start of normal feeding.

This early exposure to seawater of the larval intestine is also the most likely explanation of the important increase of the inorganic content, immediately after transition from N_5 to PZ_1.

Lipids are the most important energy sources for eggs and nonfeeding larvae, and their composition and availability have been associated with the success of hatching, survival and moulting (Lavens and Sorgeloos, 1991; Ouellet *et al.* 1992; Palacios *et al.* 1998, 1999). According to our results, all organic fractions are used as substrates, but proteins and carbohydrates decline at rates that are similar to that of individual organic weight, with mean global losses of 23, 20 and 25%. In contrast, the final lipid content was 47% of the initial value, which confirms its preferential consumption during the early stages of larval development of *L. vannamei*.

However, in agreement with the results by Lemos and Phan (2001) with *F. paulensis* larvae, proteins also play a significant role. In their case, the energy content calculated from the caloric equivalents of the three main organic components was consistently lower than those obtained by wet combustion, but showed similar trends, that coincides with our results.

According to our data, there was a significant correlation between the two sets of values ($r = 0.806$; $p < 0.01$), but those calculated using the caloric equivalents Gnaiger, (1983) were between 12 and 22% lower than those obtained by direct calorimetry, probably as a result of the presence of reserves of low molecular weight such as free aminoacids, that were not detected by our analytical techniques (Table 3).

Table 3. Protein, carbohydrate and lipid contents (in µg/ind) of *Litopenaeus vannamei* during lecithotrophic development. E = energy content (in mJ/ind) calculated with the caloric equivalents of each organic constituent (Gnaiger, 1983). Σ = total energy content. R = ratio between energy calculated and obtained by direct calorimetry.

	E	N_1	N_3	N_5	PZ_1
Protein	1.38	1.33	1.26	1.08	1.06
Carbohydrate	0.10	0.09	0.09	0.07	0.08
Lipid	1.20	0.90	0.86	0.71	0.56
E (Prot.)	32.64	31.45	29.80	25.54	25.07
E (Carb.)	1.72	1.54	1.54	1.20	1.37
E (Lip.)	47.47	35.60	34.02	28.09	21.99
Σ	81.83	68.59	65.36	54.83	48.43
R	0.779	0.786	0.833	0.786	0.880

However, the contribution of 7.57 mJ/ind due to protein consumption represents 22.6% of the calculated energy loss and 15.1% of that measured directly, showing that also proteins are used as a metabolic substrate by the lecithotrophic larvae of *L. vannamei,* and most probably of other species of penaeid shrimp.

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