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Relation between lipid and fatty acid composition of eggs and larval survival in white pacific shrimp (Penaeus vannamei, Boone, 1931)

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Abstract: In this study, lipid classes and fatty acid composition were determinedin eggs of P. vannamei as a function of survival to zoeaIII stage. Spawns were reared individually to zoea III and grouped, accordingtotheir final survival to this larval stage, into spawns of high and lowsurvival.Eggs of individual spawns were analyzed for lipid and fatty acid composition ofneutral and polar lipids and the results were then grouped according tosurvivalto zoea III. The lipids within each group (high and low survival to zoea III)were pooled for the separation of phospholipids and the analysis of their fattyacid composition.Higher levels of triglycerides, carotenoids, and linoleic (18:2n-6) acid ineggs were associated with improved survival to zoea III. Linoleic acid washigher in spawns from the high survival group in both neutral and polarfractions and in most of the phospholipid classes analyzed. Docosahexanoic acid(DHA) was not related to survival to zoea, probably because its content washighenough (> 15% of total fatty acids) to satisfy embryo and early larvaldevelopment needs. A high content of eicosapentaenoic acid (EPA) was found inphosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine, andphosphatidylinositol compared to other phospholipid classes, suggesting aspecific role of EPA in these lipids. These results describe the specificphospholipid composition of penaeid eggs and could (potentially) be used aspredictive indicators of larval quality for research and production purposes.

Keywords: Carotenoid - Larval culture - Larval quality - Phospholipid - Triglycerides

Abbreviations

AA = arachidonic acid
DHA = Docosahexanoic acid
EPA = eicosapentaenoic acid
GPL = glycolipids, phosphatidic acid, phosphatidyl glycerol and diphosphatidyl glycerol
HUFA = highly unsaturated fatty acids
LPC = lysophosphatidylcholine
LPCb = lyso-2-phosphatidyl choline
LPE = lysophosphatidylethanolamine
LPEb = lyso-2-phosphatidyl ethanolamine
PC = phosphatidylcholine
PCPlsm = phosphatidylcholine plasmalogen
PE = phosphatidylethanolamine
PEPlsm = phosphatidylethanolamine plasmalogen
PI = phosphatidylinositol
PL = phospholipids
PS = phosphatidylserine
TG = triglycerides

Introduction

The influence of the broodstock diet on the quality of the eggs and early larval stages has been well documented for Crustacean maturation (Millamena, 1989; Bray *et al*., 1990; Alava *et al*., 1993; Xu *et al*., 1994; Cahu *et al*., 1995; Marsden *et al*., 1997; Cavalli *et al*., 1999; Wouters *et al*., 1999). In these studies, several diets are offered to the broodstock, and their effect evaluated on the offspring from a biochemical (i.e. fatty acid composition) or production (i.e. fecundity, hatching rate) point of view. In commercial hatcheries, a broodstock diet that has proven to yield good results is kept constant, as are the rearing conditions that are maintained with the minimum possible variation. However, even under stable conditions, there are significant differences in the biochemical composition of eggs (Palacios *et al*., 1999). Differences between eggs, under otherwise similar conditions, have also been documented for turbot (Rainuzzo *et al*., 1997). This indicates variations in the quality and quantity of nutrients transferred from the spawners to the eggs. The quantity and quality of the nutrients in eggs can have an influence on further embryonic and larval development (Lavens and Sorgeloos, 1991; Xu *et al*., 1994; Wickins *et al*., 1995; Palacios *et al.*, 1999). It is not clear how the particular nutrients present in the eggs are related to further development. For production, it would be of interest to measure one or several variables directly in the egg, and to relate them to one or more production variables at a later stage. One variable that has been proposed as a possible predictive criterion is the content of lipid reserves in the eggs (Fraser, 1989). Lipids have energetic and structural functions during embryonic and early larval development, a period in which they are gradually used and thus depleted (Cahu *et al*., 1988; Mourente *et al*., 1995). Lipid content in eggs

can determine early larval success for survival and optimal development, currently referred to as larval condition or quality. Quantitatively, triglycerides (TG) are the most important lipids for storing energy, and their content has been associated with the physiological condition of different larvae (Fraser, 1989; Ouellet *et al*., 1992). In addition to TG, the performance of the larvae has been associated with phospholipids (PL) with specific functions (Harrison, 1990; Coutteau *et al*., 1997). In particular, the fatty acid composition of TG and PL have been shown to be related to fecundity, fertilization, and hatching rates (Xu *et al.*, 1994; Cavalli *et al*., 1999). Our objective was to measure the general lipid profile and fatty acid composition in shrimp eggs in an attempt to find indicators that would predict the performance of larvae at later stages of development.

Material and methods

Individual spawns were obtained from a commercial hatchery (Acuacultores de La Paz, S.A., Mexico) under the standard conditions previously described (Palacios *et al.*, 1999). In brief, females were subject to eyestalk ablation and stocked at a density of six animals per m^2 and a 1.5:1 male to female ratio with 200% daily water exchange, 28 °C and 36‰ (average temperature and salinity). The diet was composed of 40% squid, 15% polychaetes, 40% clams, and 5% of a commercial maturation diet (Rangen Inc., Idaho, USA) divided into five daily rations accounting for a total daily supply of 10% of shrimp biomass. Mature females with an attached spermatophore were placed in individual 160-L spawning tanks in the evening. After spawning, the number of eggs per spawn (fecundity) was estimated by stirring the spawning tanks and counting three 5-mL subsamples. Fertilization rate was estimated from three additional subsamples taken 12 h after spawning. For biochemical analyses, approximately 100-mg samples of freshly spawned eggs (in the first and second cellular cleavage) from each spawn were concentrated using a 75-μm mesh sieve, filtered under vacuum, collected in a 5-μm Millipore filter and frozen at -70 °C until further analysis. All spawns were hatched individually. The number of viable nauplii per spawn was estimated after positive phototropism selection, and approximately 30 nauplii at stage IV were collected from each spawn in 4% formaldehyde to measure rostrocaudal length by using a light microscope and micrometer.

Spawns were individually reared to zoea III stage in 80-L conical fiberglass tanks at a density of 100 nauplii per L, 28 °C, and 36‰ (average temperature and salinity). During larval culture, survival to zoea I, II, and III was estimated. The diet offered from

nauplii V stage was composed of an algal mixture of *Chaetoceros muelleri*, *Tetraselmis suecica*, *Isochrysis galbana*, and *Thalassiosira pseudonana* administered with a continuous feeding system combined with a daily water exchange of 30% (Ramírez *et al*., 1999). The larvae reared individually, each from different females, were assigned to two groups according to their final survival to zoea III: high survival (> 80%, n = 4 individual spawns), and low survival (< 60%, n = 5 individual spawns).

Lipids were extracted with chloroform: methanol (2:1) according to Bligh and Dyer (1959) and the lipid extract was stored at –20 °C in chloroform under a nitrogen atmosphere until further analysis. General lipid composition was measured with a Iatroscan TLC/FID MK-5 analyzer and chromarods S-III, which were previously brought to a constant humidity in a hydration chamber (Ackman and Heras, 1997). One μL of sample was spotted on each rod and lipid classes were developed for 35 min in a mobile phase of hexane: ethyl acetate: diethyl ether: formic acid (90:7:3:1) (Ackman and Heras, 1997). Rods were scanned at a hydrogen flow rate of 160 mL/min, airflow of 2000-mL min^{-1}, and a scanning speed of 30-mm min^{-1}. Lipid classes were identified by comparison of retention times against appropriate standards (Sigma, St. Louis, USA), and concentration was calculated from the peak area of each standard.

Neutral and polar lipid fractions were separated on a silica-gel microcolumn, and collected in vials containing an internal standard (23:0) and butylated hydroxytoluene (BHT). Fatty acids were transesterified by boron-trifluoride-methanol (BF_3 14% methanol, Supelco) and treated as described by Marty *et al*. (1992). The fatty acid methyl esters were analyzed in a Chrompak 9001 GC equipped with a DBWAX (25 m x 0.32 mm, 0.2-um film thickness) capillary column, a flame ionization detector, hydrogen

as the carrier gas, and a temperature gradient from 150 to 250 °C at 3 °C min^{-1}. The fatty acids were identified by comparing their retention times with those of standards.

For the analyses of phospholipid classes, individual samples were pooled and separated by HPLC using a combination of two columns (Merck-Hitachi system) with an UV detector at 206 nm as described by Soudant *et al*. (1995). Each phospholipid class was collected in vials containing 23:0 as an internal standard and BHT and treated as described above. The lyso fractions (lysophosphatidylcholine or LPC, and lysophosphatidylethanolamine or LPE) reported in the present study could correspond to two lyso forms; the lyso-1-phosphatidyl resulting from hydrolysis during separation of the alkenyl forms of phosphatidylcholine (PC) or phosphatidylethanolamine (PE), named plasmalogens (PCPlsm, PEPlsm), and the biogenic lyso-2-phosphatidyl (LPCb and LPEb), as previously indicated by Soudant (1995) and Soudant *et al*. (1995). The exact nature and concentration of each lyso-1 or lyso-2 form are currently under investigation.

Total proteins were determined by the Coomassie blue method (Bradford, 1976) after digestion of the crude homogenate with 0.1 N NaOH. Total carotenoids were extracted with acetone: methanol (2:1) for 24 hours at 4 °C. After extraction, samples were centrifuged at 10,000 *g* and absorbance of the supernatant was recorded at 470 nm. Total carotenoid concentration was calculated according to Strickland and Parsons (1972).

Data are presented as mean ± standard deviation (*s*). One way ANOVA was used to assess significant differences at the level of $P < 0.05$. Percentage data were transformed to arcsine values for statistical analysis. For the fatty acid comparison of each phospholipid class only one data set for each class is reported.

Results

The cumulative survival throughout larval development to zoea III was 85% for the batches assigned to the high survival group, and 38% for the spawns in the low survival group (Table 1). No significant differences were observed in the spawner body weight, number of eggs and nauplii, nauplii length, and fertilization and hatching rates. No significant differences were observed in the survival to early zoea stages, although a decline can be noted at the zoea II stage (Table 1).

Triglycerides were 21% higher in eggs from the high survival group compared to the level in the eggs of the low survival group (Table 2). Total carotenoids were 34% higher in eggs from the high survival group (Table 2). No significant differences were observed for cholesterol esters, free cholesterol, total phospholipids, total protein content, or total lipids, although total lipids tended to be higher in eggs from the high survival group ($P<0.12$).

The proportion of fatty acids in the neutral and polar lipid fraction are presented in Table 3. In general, the polar lipid fraction had a higher proportion of 18:0 and 20:5n-3 (eicosapentaenoic acid; EPA), and a lower proportion of 14:0 and 16:0 fatty acids compared to the neutral lipid fraction. These variations were independent of the survival rate to zoea III. Linoleic acid (18:2n-6) was higher in eggs from the high survival group in the neutral lipid fraction, and a similar, although not significant, trend was observed for this fatty acid in the polar fraction. The 18:2n-6 constitutes 55% to 62% of the (n-6) fatty acids in the polar fraction and 61% to 71% in the neutral fraction, rendering the sum of (n-6) PUFA significantly higher for the high survival group in both fractions.

As mentioned in the methodology section, it is not possible to compare phospholipid

levels or their fatty acid composition between the high and low survival groups because the eggs were pooled for the analyses. Nevertheless, tables 4 and 5 include these results for description purposes in shrimp eggs.

The concentration of each phospholipid class was calculated from the fatty acid content of each fraction, and is reported both in proportion to total phospholipids and as concentration in eggs (Table 4). PC was the major phospholipid followed by LPC (both LPCb and PCPlsm forms), with the other classes at levels lower than 10%. Thus, the phospholipids containing choline (LPC and PC) represented a total of 83-86%, and those containing ethanolamine summed 11% (LPE and PE).

Table 5 depicts the proportion of fatty acids in each phospholipid class. In a general way, the proportions of fatty acid present in PC and LPC were similar to that of the total polar lipid fraction (See Table 3). The proportion of 20:4n-6 in the PI was two to five times higher in comparison to other phospholipid classes, and therefore, total (n-6) fatty acid percent was higher in this class. The proportion of 20:5n-3 was higher in the PE, LPE, PI and PS classes in comparison to other phospholipid classes. The proportion of 14:0 in the non-separated fraction, corresponding to glycolipids, phosphatidic acid, phosphatidyl glycerol, and diphosphatidyl glycerol classes (GPL), and of 18:0 in the PI, PE, PS, and the GPL were higher in comparison to other phospholipid classes. The PS, PI, and GPL had a higher proportion of total saturated fatty acids, PC and LPC classes had more monoenes, whereas PE and LPE classes had higher levels of total polyenes and total (n-3) fatty acids.

Discussion

In the present study, eggs with higher TG levels resulted in a higher survival to zoea III, indicating that differences in egg TG content are related not only to the survival throughout embryonic and early larval development but until the zoea stages. Thus, it seems that the importance of the initial levels of TG in eggs is not only related to the stages where the larvae are lecitotrophic, but also to later stages of larval development, which feed on exogenous sources. The influence of egg lipid reserves on the hatching success has been well documented (Fraser, 1989; Cahu *et al.*, 1988; Ouellet *et al.*, 1992; Mourente *et al.*, 1995; Palacios *et al.*, 1999). However, in this study, the egg TG content did not influence the hatching rate. The effect of previous reserves on the feeding of Penaeid larvae was described by Mourente *et al.* (1995), who reported a decrease in TG content from mysis to postlarvae in *Penaeus kerathurus*, because of the use of this lipid for energy purposes to satisfy a sort of substarving condition. In other decapod crustaceans, the nonfeeding larval stages, such as puerulus of the lobster *Panulirus cygnus* (Lemmens, 1991) or megalopa of the hermit crab *Pagurus bernhardus* (Anger, 1989), depend on nutrients accumulated from earlier feeding stages. This dependence was termed secondary lecitotrophy by Anger (1989), in contrast to primary lecitotrophy, which refers to the role of maternal reserves for the development of postembryonic stages, and which correspond to nauplii stages in Penaeid shrimp. Thus, the substarving condition described for penaeid mysis and postlarvae (Mourente *et al.,* 1995) might correspond to a secondary lecitotrophy, in which there is a partial reliance on reserves obtained from food during zoea stages and accumulated for later use during mysis stages. The reliance of feeding-stage larvae on maternal reserves is also

supported indirectly by other studies. Spawns produced by broodstock in poor physiological condition had a lower TG content in eggs and nauplii, (Palacios *et al*., 1998), which resulted in larvae with lower survival rates to later stages (Palacios *et al*., 1999). Furthermore, postlarvae reared from nauplii with low TG levels also had a lower tolerance to adverse environmental conditions, such as low salinity levels (Palacios *et al.*, 1999). A similar result was obtained for *Macrobrachium rosenbergii* in which larvae already in feeding stages obtained from females fed high levels of (n-3) and (n-6) fatty acids had higher tolerance to high ammonia concentrations (Cavalli *et al.*, 1999). A progressive decrease in neutral and polar lipid fractions was observed during zoea development through its three substages (Cahu *et al*., 1988). The long-term effect of egg lipid reserves on the zoea larvae could either be explained by the mere use of these reserves to this advanced stage (due to a substarving-like period or a low capacity of food transformation), or because the reserves determined a faster development rate of previous stages, or even that they provided a better overall condition, thus increasing their endurance and survival.

When analyzing specific phospholipid classes, we observed that PC was the main phospholipid followed by LPC, compared to other classes that represent less that 10% of total phospholipids. As was mentioned in the methodology section, the LPC corresponds to LPCb and to PCPlsm. It has been reported that LPCb or the PCPlsm forms do not represent an important phospholipid in crustaceans or in other marine invertebrates (Sargent, 1989; Vaskovsky, 1989). However values of LPC (probably LPCb, the authors did not specify) from 6% (Mourente *et al*., 1991) up to 40% (Teshima *et al*., 1988) of total polar lipid content in ovaries have been reported for Penaeid

shrimp. In another study undertaken by our group, its presence in large amounts in eggs of *P. vannamei* was confirmed and its variation (and thus its possible role) during early larval development is being assessed.

Fatty acids, particularly highly unsaturated forms (HUFA), such as eicosapentaenoic acid (EPA) or docosahexaenoic (DHA), represent the most investigated components in relation to spawn and larval quality, regardless if they originate from broodstock or larval diet. Middledich and Missler (1980) first established the importance of HUFA in penaeid shrimp reproduction. Further studies have shown that high levels of HUFA in broodstock diet are associated to higher values of spawn quality such as fecundity, fertilization, and hatching (Millamena, 1989; Xu *et al.*, 1994; Cahu *et al.*, 1995). However, other studies have failed to attribute a better reproductive performance and resulting spawn quality to HUFA content in broodstock diet (Luis and Ponte, 1993; Marsden *et al.*, 1997). During early larval nutrition, HUFA enrichment of *Artemia* and rotifers in the diet of zoea II stage does not improve larval survival, development, and performance (Wouters *et al*., 1997). However, these authors considered the possibility that the essential role of HUFA in these stages had been masked by high levels of HUFA supplied in algae before the zoea II stage (Wouters *et al.*, 1997), and possibly also to the remaining HUFA reserves from the vitellus or yolk. In this sense, the present study examined if there were some particular fatty acids in eggs that could be associated more closely to the survival to zoea III stage. EPA content in total neutral and polar lipid fractions was slightly lower in eggs with low survival, but this difference was accentuated when analyzing EPA in specific phospholipid classes, mainly in LPE (half that of the high survival group), and to a lesser extent in PS, PI, and

GPL. A specific role of EPA in these PL classes (principally LPE) is supported by its higher content when compared to PC and LPC. This characteristic has been observed in the plasmalogens of human origin (Thomas *et al*., 1990) and also in the scallop *P. maximus* (Soudant *et al*., 1995).

A surprising result is that DHA levels in total neutral and polar lipids or in each phospholipid class were not different between batches with high or low survival. These results contrast to the well-documented role of DHA in embryogenesis and early larval development of Penaeid shrimp (Millamena, 1989; Xu et al., 1994; Cahu et al., 1995). However, in this study the proportion of DHA was high (15% or more), which could explain why it did not seem to have a critical role. Indeed, Cahu and coworkers (1995) showed that at certain levels of DHA in the diet (above 10% of total fatty acids), eggs are able to incorporate DHA at levels of 13% to 15% of total fatty acids, a level high enough for adequate hatching.

In contrast, the level of 18:2n-6 was higher in eggs with high survival to zoea III. As a consequence, the high survival group had significantly higher levels of total (n-6) fatty acids in both the neutral and the polar fraction. High levels of 18:2n-6 fatty acids have sometimes been associated with a poor diet (Watanabe *et al*., 1984a; Cahu *et al.*, 1994). However, Luis and Ponte (1993) found that the beneficial effects of supplying broodstock diet with polychaete of the genus *Nereis* could be related to their high content in these fatty acids, especially 18:2n-6 fatty acids. Similarly, Cavalli *et al.* (1999) fed *Macrobrachium rosenbergii* broodstock with a diet high in 18:2n-6 and obtained increased fecundity. The exact significance of these results in terms of the role played by these fatty acids during early larval development should be further investigated.

Soudant (1995) described a characteristic distribution of fatty acids between the different phospholipid classes for the scallop *Pecten maximus* larvae. Although some differences between classes and survival groups can be observed, we did not obtain such a clear distribution of fatty acids for *Penaeus vannamei* eggs using the same separation system. This could point to differences in the accumulation and use of the fatty acid in the different phospholipid classes in different groups of marine invertebrates.

Lower carotenoid levels were found in eggs from the group with low survival to zoea III stage. Similarly to TG, carotenoids may be of importance during secondary lecitotrophy throughout embryonic development to zoea III. In a previous work, we observed that the physiological condition of spawners affected the carotenoids levels in eggs and nauplii, which in turn were associated with larval survival and performance (Palacios *et al.*, 1999). The beneficial role of carotenoids was also shown by a higher survival to zoea II, when paprika was supplemented in the broodstock diet (Wyban *et al*., 1997). In eggs, the importance of carotenoids is probably related to vitamin A production, a precursor of retinoic acid, which plays an important role in early differentiation. They can also protect unsaturated lipids against oxidation (Harrison, 1990), and may have a role in egg buoyancy (Watanabe *et al*., 1984b). Levels of carotenoids in crustaceans decrease during development, which also suggests their use or oxidation for specific physiological purposes (Petit *et al*., 1991; Mantiri *et al*., 1996; Palacios *et al.*, 1999). Their role as a natural antioxidant could explain why levels of HUFA are not so directly related to survival to zoea III, provided enough carotenoids are present to prevent HUFA oxidation. This possibility is supported by the inclusion of α-

tocopherol in broodstock diet to prevent oxidation of HUFA when the levels of these fatty acids are increased in the diet (Cahu *et al.*, 1995).

In summary the present study indicates that some specific lipid components and carotenoids could be used as predictive indicators of larval quality for experimental and production purposes.

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Table 1. Production variables (means ± *s*) of individual spawns of *P. vannamei* grouped according to their cumulative survival through culture to zoea III.

Survival to zoea III	High	Low	
Spawner body weight (g)	40 ± 7	38 ± 2	N.S.
Eggs per spawn ($x10^3$)	213 ± 114	218 ± 62	N.S.
Fertilization (%)	79 ± 15	72 ± 14	N.S.
Nauplii per spawn ($x10^3$)	105 ± 44	118 ± 30	N.S.
Hatching rate (%)	57 ± 33	58 ± 27	N.S.
Nauplii length (μm)	364 ± 21	342 ± 23	N.S.
Survival to Zoea I (%)	97 ± 4	91 ± 13	N.S.
Survival to Zoea II (%)	92 ± 10	64 ± 34	N.S.
Survival to Zoea III (%)	85 ± 10	38 ± 29	$P < 0.05$

Table 2. General biochemical composition (means ± *s*) in individual spawns of *P. vannamei* grouped according to their cumulative survival through culture to zoea III.

	High	Low	
Cholesterol esters (mg g^{-1})	1.2 ± 0.2	2.1 ± 1.1	N.S.
Triglycerides (mg g^{-1})	30.0 ± 2.8	23.3 ± 3.9	$P < 0.05$
Free cholesterol (mg g^{-1})	0.9 ± 0.4	0.7 ± 0.3	N.S.
Phospholipids (mg g^{-1})	21.7 ± 2.6	18.7 ± 4.1	N.S.
Total lipid (mg g^{-1})	53.9 ± 3.6	46.1 ± 8.4	N.S.
Total carotenoid (μg g^{-1})	61.5 ± 13.4	40.6 ± 9.8	$P < 0.05$
Total protein (mg g^{-1})	153 ± 38	137 ± 16	N.S.

All concentrations are reported on a wet weight basis

Table 3. Fatty acids (mol %) (means ± *s*) in the polar and neutral fraction in eggs from individual spawns of *P. vannamei* grouped according to their cumulative survival through culture to zoea III.

	Polar Lipids		Neutral Lipids	
	High	Low	High	Low
14:0	1.1 ± 0.2	1.5 ± 0.1	3.9 ± 0.3	4.0 ± 0.2
16:0	22.4 ± 0.8	24.5 ± 2.3	29.2 ± 1.2	31.8 ± 2.0
18:0	10.4 ± 0.7	10.0 ± 0.6	4.6 ± 0.4	4.8 ± 0.6
16:1n-7	5.7 ± 0.6	6.2 ± 0.4	7.8 ± 0.4	8.1 ± 0.6
18:1n-9	17.0 ± 2.3	16.4 ± 1.5	15.7 ± 2.7	14.3 ± 1.2
18:1n-7	3.8 ± 0.5	3.6 ± 0.3	3.4 ± 0.5	3.2 ± 0.2
20:1n-9 + n-7	3.7 ± 0.4	3.8 ± 0.4	2.4 ± 0.2	2.6 ± 0.3
18:2n-6	3.7 ± 1.4	2.7 ± 0.7	3.8 ± 1.6	2.5 ± 0.6*
18:3n-3	0.4 ± 0.1	0.4 ± 0.04	0.5 ± 0.1	0.6 ± 0.2
18:4n-3	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
20:2n-6	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
20:4n-6	2.0 ± 0.2	1.8 ± 0.3	1.3 ± 0.1	1.3 ± 0.2
20:5n-3	11.0 ± 0.6	10.1 ± 1.7	8.5 ± 0.8	7.7 ± 1.1
22:5n-3	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.1
22:6n-3	15.5 ± 3.0	15.6 ± 2.5	15.8 ± 3.2	15.9 ± 2.3
Σ Saturates	33.9 ± 0.1	35.9 ± 2.9	37.7 ± 1.0	40.7 ± 2.7
Σ Monoenes	30.2 ± 2.0	30.0 ± 1.7	29.3 ± 2.9	28.2 ± 1.9
Σ Polyenes	34.6 ± 1.9	32.5 ± 4.0	31.69 ± 2.0	29.75 ± 2.9
Σ (n-6)	6.4 ± 1.4	5.3 ± 0.4*	5.6 ± 1.6	4.2 ± 0.5*
Σ (n-3)	28.2 ± 3.3	27.2 ± 4.1	26.1 ± 3.2	25.5 ± 3.2
(n-3)/(n-6)	5.0 ± 1.9	5.6 ± 1.3	5.1 ± 2.4	6.1 ± 1.3

Values marked with (*) are significantly different ($P<0.05$)

Table 4. Phospholipid content of eggs from individual spawns of *P. vannamei* pooled according to their cumulative survival through culture to zoea III. Values are expressed as proportion of total phospholipids (mol %), and as concentration of each phospholipid class (mg g^{-1}).

	Percent of phospholipids		mg g^{-1} of eggs	
	High	Low	High	Low
PC	53.7	68.0	11.7	12.7
LPC	32.5	14.8	7.1	2.8
LPE	7.5	7.3	1.6	1.4
PE	3.3	3.6	0.7	0.7
PS	1.4	3.6	0.3	0.7
PI	1.2	2.3	0.3	0.4
GPL	0.52	0.48	0.11	0.09

PC = phosphatidylcholine, LPC = lysophosphatidylcholine, PE = phosphatidylethanolamine, LPE = lysophosphatidylethanolamine, PS = phosphatidylserine, PI = phosphatidylinositol, and GPL = fraction corresponding to glycolipids, phosphatidic acid, phosphatidyl glycerol and diphosphatidyl glycerol.

Table 5. Fatty acids (mol %) in phospholipids of eggs from individual spawns of *P. vannamei* grouped according to their cumulative survival through culture to zoea III.

	PC		LPC		PE		LPE		PS		PI		GPL	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
14:0	1.6	1.8	1.1	1.0	0.9	1.1	2.3	3.0	2.0	3.7	1.5	1.8	5.2	4.3
16:0	24.6	24.9	21.4	20.5	16.9	16.5	23.2	24.7	17.0	17.7	17.1	18.3	21.4	27.2
18:0	9.1	9.1	9.5	12.7	17.2	17.9	5.2	14.4	23.6	31.1	18.0	23.9	17.5	20.5
16:1n-7	6.8	7.2	5.6	5.1	3.3	2.4	8.6	4.2	2.1	1.6	2.9	1.8	6.5	8.3
18:1n-9	17.7	17.1	20.7	15.3	10.5	8.2	6.5	7.2	7.7	6.4	11.2	5.7	7.8	8.5
18:1n-7	3.8	3.8	3.7	3.2	3.2	2.9	1.6	2.7	2.3	2.3	3.0	3.1	6.0	2.7
20:1	2.6	2.9	3.0	2.9	2.1	1.9	0.5	3.5	2.1	1.3	1.6	3.4	3.2	3.2
18:2n-6	4.1	2.5	4.0	2.1	2.8	1.6	3.4	1.6	1.8	1.3	3.3	1.5	4.0	3.0
18:3n-3	0.48	0.4	0.4	0.4	0.5	1.6	0.6	0	0	0	0	0	0	0
18:4n-3	0.28	0.2	0.2	0.6	0	0	0	0	0	0	0	0	0	0
20:2n-6	0.65	0.7	0.8	0.6	0.7	1.4	0.4	1.9	0	0	0.8	1.7	0	0
20:4n-6	1.8	1.7	1.9	2.7	4.4	4.7	3.2	3.2	4.1	3.0	6.3	10.7	4.8	3.4
20:5n-3	9.8	9.4	10.2	11.2	20.6	19.6	27.1	15.5	18.4	10.6	19.6	15.4	13.0	7.8
22:5n-3	0.9	0.8	0.9	1.1	1.0	1.5	1.2	1.3	1.2	1.1	1.1	1.4	0	0
22:6n-3	13.2	15.1	14.3	17.1	16.0	17.7	15.7	14.8	16.4	15.3	12.2	10.7	10.4	11.2
Σ Saturates	35.3	35.7	32.0	34.2	35.0	35.4	30.7	42.1	42.6	52.5	36.6	44.0	44.2	52.0
Σ Monoenes	32.0	32.1	34.1	28.1	19.1	16.5	17.7	17.5	14.2	11.7	18.6	13.9	23.6	22.6
Σ Polyenes	31.2	30.7	32.7	35.7	46.0	48.1	51.5	38.3	41.9	31.2	43.3	41.4	32.3	25.4
Σ (n-6)	6.5	4.8	6.7	5.3	7.9	7.7	7.0	6.6	5.9	4.3	10.3	14.0	8.9	6.4
Σ (n-3)	24.7	25.8	26.0	30.4	38.0	40.5	44.6	31.7	36.1	26.9	33.0	27.5	23.4	19.0
(n-3)/(n-6)	3.8	5.4	3.9	5.7	4.8	5.3	6.4	4.8	6.2	6.3	3.2	2.0	2.7	3.0

See Table 4 for abbreviations