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# Quality Evaluation of Four Sources of Brine Shrimp *Artemia* spp.[1]

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## Abstract

**A study was conducted to compare fatty acid composition, hatching quality, and size of nauplii from four commercial sources of brine shrimp (*Artemia* spp.) cysts: China (CH), Colombia (COL), Great Salt Lake (GSL), and San Francisco Bay (SFB). The CH brine shrimp had a comparatively high percentage of 20:5(n-3) fatty acid (eicosapentaenoic acid or EPA), an essential fatty acid for most larval fishes, which was 10.4% of the total lipids. The COL, GSL, and SFB sources of cysts contained comparatively low percentages of EPA, 2.9, 1.2, and 1.6%, respectively. Hatching quality was determined by hatching cysts in salt (NaCl) water with a specific gravity of 1.02 at 27 C for 42 h. The COL cysts had the fastest hatching rate, with 50% of the cysts hatching in 13.4 h, while CH cysts hatched at the slowest rate, requiring 25.6 h for 50% of the cysts to hatch. Total percentage hatch was not significantly different among the SFB, COL, and GSL sources, with an average hatching percentage of 84.5, while CH cysts had a significantly lower total percentage hatch of 67.5. The SFB source produced the greatest number of nauplii ($1.6 \times 10^5$ per g of cysts) with the smallest length (382 μm), while CH produced the smallest number ($8.7 \times 10^4$) with the greatest length (500 μm). These results indicate that there is great variation in nutritional quality, hatching quality, and size of nauplii among commercial sources of brine shrimp cysts, and each of these criteria should be considered in selecting brine shrimp in a development of a feeding strategy for larval culture of a particular species.**

Prepared diets that have been developed for the larval culture of many species of fishes have not been completely effective, so live foods continue to be used. Brine shrimp nauplii, *Artemia* spp., comprise an estimated 85% of all live foods fed to larval fishes worldwide (Sorgeloos 1980). Brine shrimp cysts are commercially available from many different sources in North and South America, Europe, Australia, and Asia. Variation in nutritional quality among sources of brine shrimp has been clearly demonstrated (Beck et al. 1980; Fujita et al. 1980; Vos et al. 1984) and is discussed in detail in a review by Leger et al. (1986). Watanabe et al. (1980) with red sea bream, *Pagrus major*, and Webster (1989) with striped bass, *Morone saxatilis*, found that these differences are principally due to differences among sources of brine shrimp in the essential fatty acid, eicosapentaenoic acid (20:5(n-3) or EPA). Variation in hatching quality of the cysts and size of the nauplii has also been observed among sources of brine shrimp. Percentage of cysts hatching in a defined time period (Sorgeloos and Persoone 1975), number of nauplii produced per g of cysts (Sorgeloos et al. 1978), and weight of nauplii produced per g of cysts (Vanhaecke and Sorgeloos 1983) are criteria used to described hatching quality of brine shrimp cysts. In this study, nutritional and hatching quality among four commercial sources of brine shrimp cysts were investigated with regard to fatty acid composition, hatching rate, number of hatched nauplii per g of cysts, and size of nauplius.

## Materials and Methods

Cysts from four sources of brine shrimp were evaluated. They were San Francisco Bay (San Francisco Bay Brands, Newark, California; case 65034, lot 1106), Colombia

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(Aquarium Products, Glen Burnie, Maryland; lot 190), China (Biomarine, Inc., Hawthorne, California; can 686523), and Great Salt Lake (Biomarine, Inc., Hawthorne, California; can 686038).

### *Hatching Quality*

The nauplii were hatched in 56 L transparent plastic conical hatching containers (Chem-Tainer Industries, Inc., Babylon, New York) containing 48 L of aerated salt (NaCl) water (30 mg/1,000 mL). One gram of each source of cysts per L of salt water was placed into duplicate hatching containers. Temperature in the hatching room was 27 C and continuous illumination was provided by fluorescent ceiling lights (approximately 1,000 lux). At 3 h intervals, two 2 mL samples of water containing suspended materials were taken from each container with a pipette, and the numbers of nauplii and unhatched cysts were counted until 42 h of incubation. Percentage hatch was calculated by dividing the number of nauplii by the number of unhatched cysts plus the number of nauplii and multiplying by 100. After 42 h, total nauplii produced per g of cysts were determined. Hatched nauplii were collected from each hatching container by stopping the aeration and allowing the nauplii to settle to the bottom of the container and subsequently transferring them to a graduated cylinder. The nauplii were allowed to settle, and the volume of nauplii was recorded. Two 1 mL samples of nauplii were removed with a pipette, and the number of nauplii in each was counted in a 1:100 dilution. The average number was used to calculate the number of hatched nauplii per g of cysts. Naupliar size (length) was determined by measuring 10 nauplii under a dissecting microscope with an ocular micrometer.

### *Lipid Analysis*

Total lipids were extracted from 2 g of nauplii collected after 30 h incubation under the hatching conditions described above. After extraction with methanolchloroform, as described by Bligh and Dyer (1959), the lipids were dried by sparging with nitrogen. The fatty acid fraction was separated from the sterol fraction by alkaline hydrolysis (0.4 mL 33% KOH in 4 mL ethanol) for 120 min at 90 C (Kates 1986). The hydrolysate was washed three times with 4 mL hexane to remove sterols and then acidified (pH 1–2) with 6 N HCl. Fatty acids were obtained by washing three times with 4 mL hexane and converted to their methyl ester derivatives using $BF_3$/methanol (Morrison and Smith 1964). The fatty acid methyl esters were analyzed using a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector and a 30 m capillary column (0.25 mm internal diameter) coated with SP-2330 (Supelco, Inc., Bellefonte, Pennsylvania) (Weete et al. 1983). The carrier gas was nitrogen at a column pressure of 0.58 kg/cm$^2$. Injector and detector temperatures were 300 C and the oven temperature was programmed from 140 to 210 C at 4 C/min. Detector response was recorded and quantitated with a Hewlett-Packard 3380A integrator-recorder. An internal standard, 24:1(n-9) (nervonic acid) was added, and the fatty acid methyl esters were identified by comparison of their retention times with those of authentic standards (Nu-Chek Prep, Inc., Elysian, Minnesota).

### *Data Analysis*

Analysis of variance was used to determine if differences existed among treatment means for percentage of fatty acids, hatching percentage, and number of nauplii per g of cysts, using the ANOVA procedure of SAS (Statistical Analysis Systems 1985). Fatty acid and hatching percentages were normalized by arcsine transformation prior to analysis (Zar 1984). When a significant difference was indicated, Duncan's multiple range test was used to determine significant differences between means. To compare hatching rates, percentage hatch was regressed on incubation time by the SAS non-

TABLE 1. *Percentages (±SE) of fatty acids in the total lipids extracted from brine shrimp nauplii from various sources.*[1]

| Fatty acid | Spurce[2] | | | |
|---|---|---|---|---|
| | CH | COL | GSL | SFB |
| 12:0 | 0.1 ± 0.1[b] | 0.1 ± 0.0[b] | 0.3 ± 0.1[a] | 0.1 ± 0.1[ab] |
| 14:0 | 1.6 ± 0.1[a] | 1.1 ± 0.2[ab] | 0.8 ± 0.1[b] | 0.8 ± 0.1[b] |
| 14:12(n-5) | 1.1 ± 0.1[a] | 1.2 ± 0.2[a] | 0.4 ± 0.1[a] | 1.4 ± 0.5[a] |
| 15:0 | 0.7 ± 0.0[a] | 0.6 ± 0.0[a] | 0.5 ± 0.0[a] | 0.7 ± 0.1[a] |
| 16:0 | 12.3 ± 0.1[a] | 13.3 ± 0.9[a] | 11.1 ± 0.7[a] | 12.8 ± 0.3[a] |
| 16:1(n-7) | 19.8 ± 0.3[a] | 4.5 ± 0.3[bc] | 3.3 ± 0.2[c] | 5.7 ± 0.7[b] |
| 16:1(n-9) | 1.1 ± 0.1[a] | 0.6 ± 0.0[b] | 0.3 ± 0.1[c] | 0.6 ± 0.0[b] |
| 17:0 | 2.1 ± 0.2[a] | 1.0 ± 0.1[b] | 1.1 ± 0.2[b] | 1.1 ± 0.1[b] |
| 18:0 | 4.3 ± 0.3[b] | 4.1 ± 0.4[b] | 7.5 ± 0.3[a] | 3.6 ± 0.2[b] |
| cis18:1(n-9) | 16.1 ± 0.5[b] | 19.8 ± 0.7[a] | 19.8 ± 0.6[ab] | 19.0 ± 1.3[ab] |
| trans18:1(n-9) | 0.4 ± 0.1[a] | 0.0 ± 0.0[b] | 0.4 ± 0.1[a] | 0.0 ± 0.0[b] |
| 18:1(n-7) | 12.0 ± 0.3[a] | 8.7 ± 0.3[b] | 8.8 ± 0.2[b] | 9.1 ± 0.2[b] |
| 18:2(n-6) | 5.1 ± 0.2[c] | 6.9 ± 0.1[b] | 8.2 ± 0.1[a] | 6.3 ± 0.4[b] |
| 18:3(n-3) | 3.6 ± 0.2[c] | 25.9 ± 0.5[ab] | 28.2 ± 0.8[a] | 24.6 ± 1.3[b] |
| 20:0 | 0.5 ± 0.1[a] | 0.5 ± 0.1[a] | 0.6 ± 0.1[a] | 0.8 ± 0.0[a] |
| 20:1(n-9) | 0.2 ± 0.2[ab] | 0.0 ± 0.0[b] | 0.6 ± 0.1[a] | 0.0 ± 0.0[b] |
| 20:2(n-6)[3] | 0.6 ± 0.1[c] | 3.3 ± 0.1[b] | 0.6 ± 0.1[c] | 4.7 ± 0.5[a] |
| 20:3(n-3) | 0.1 ± 0.0[a] | 0.1 ± 0.1[a] | 0.3 ± 0.1[a] | 0.3 ± 0.1[a] |
| 20:4(n-6) | 1.9 ± 0.2[a] | 0.8 ± 0.2[b] | 1.7 ± 0.2[ab] | 1.3 ± 0.2[ab] |
| 20:5(n-3) | 10.4 ± 0.4[a] | 2.9 ± 0.2[b] | 1.2 ± 0.4[c] | 1.6 ± 0.4[bc] |
| 22:0 | 0.1 ± 0.0[a] | 0.2 ± 0.1[a] | 0.3 ± 0.2[a] | 0.1 ± 0.1[a] |
| 22:1(n-9) | 0.1 ± 0.0[a] | 0.2 ± 0.2[a] | 0.1 ± 0.0[a] | 0.1 ± 0.0[a] |
| Unknown fatty acids | 5.8 ± 0.6 | 4.2 ± 0.3 | 3.9 ± 0.5 | 5.3 ± 0 |
| Total n-3 | 14.2 ± 0.4[b] | 28.9 ± 0.8[a] | 29.7 ± 0.9[a] | 26.5 ± 1.0[a] |
| EPA/LLA[4] | 2.9 ± 0.2[a] | 0.1 ± 0.0[b] | 0.1 ± 0.0[b] | 0.1 ± 0.0[b] |
| Total lipids | 17.2 ± 1.0[a] | 18.2 ± 3.6[a] | 16.2 ± 3.1[a] | 20.2 ± 1.2[a] |

[1] Values are means ± SE of 3 replications. Values in the same row with the same superscript are not significantly different ($P > 0.05$).

[2] Sources are Chinese (CH), Colombian (COL), Great Salt Lake (GSL), and San Francisco Bay (SFB).

[3] May be 18:4(n-3).

[4] EPA/LLA = eicosapentaenoic acid/linolenic acid.

linear procedure and the derived hatching curves were analyzed with multivariate and univariate analyses of variance techniques to determine if differences existed among the sources of brine shrimp cysts. Times for 10, 50, and 75% of the cysts to hatch were estimated from the regressions and compared with Duncan's multiple range test to determine where differences existed among the sources of brine shrimp cysts.

## Results

### *Lipids and Fatty Acid Composition*

No differences were found in the percentage of total lipids (dry basis) in the nauplii of in fatty acid methyl esters (FAME) per g of lipid among the sources of brine shrimp cysts ($P > 0.05$). However, there were major differences in fatty acid composition of the lipid among the various sources of brine shrimp (Table 1). The Chinese (CH) brine shrimp had a higher percentage of 20:5(n-3) fatty acid (eicosapentaenoic acid or EPA) ($P > 0.05$), but a lower percentage of 18:3(n-3) (linolenic acid) ($P < 0.05$) when compared to brine shrimp from the other sources, the Colombian (COL), Great Salt Lake (GSL), and San Francisco Bay (SFB). The EPA/linolenic acid ratio was much higher in the CH brine shrimp than in the other three sources ($P < 0.05$). Although the CH brine shrimp contained more EPA, they contained less total

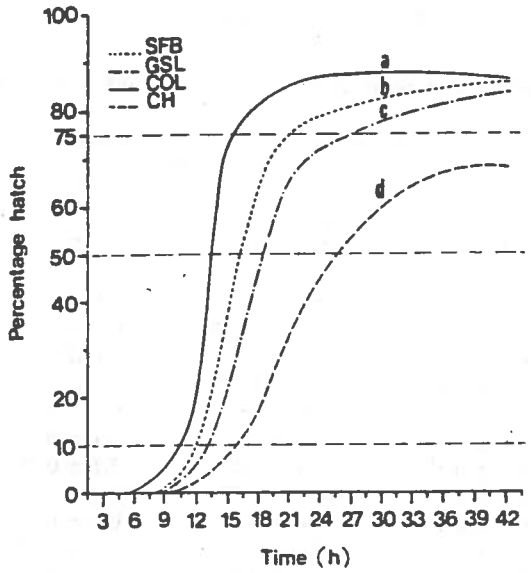


FIGURE 1. *Percentage of brine shrimp cysts hatched after various incubation periods at 27 C. Brine shrimp sources are Chinese (CH), Colombian (COL), Great Salt Lake (GSL), and San Francisco Bay (SFB). Broken horizontal lines indicate 10, 50, and 75% hatch. Hatching curves with different letters have significantly different hatching rates (P < 0.05).*

n-3 fatty acids than the other sources ($P < 0.05$).

### *Hatching Quality*

Hatching rates differed significantly among the sources of brine shrimp cysts. Hatching curves (Fig. 1) were different for each source ($P < 0.05$). The COL source began to hatch first and hatched fastest, while the CH source began to hatch last and hatched at the slowest rate. The COL cysts required only 14.9 h for 75% of the cysts to hatch, while the CH cysts required 25.6 h for only 50% of the cysts to hatch (Table 2). Total percentage hatch was not different ($P > 0.05$) in the SFB, GSL, and COL sources with an average of 84.5% of the cysts hatching, while the CH cysts had a lower total hatch (67.5%) than the other sources ($P < 0.05$).

The SFB cysts yielded the highest number of nauplii per g of cysts, which was significantly higher ($P < 0.05$) than that of the CH cysts but not that of the cysts from the other sources. The CH cysts produced the largest nauplii (500 μm), which were significantly ($P < 0.05$) larger than those from the SFB or COL cysts. The SFB nauplii (382 μm) were smaller than all other sources ($P < 0.05$).

## Discussion

There was a clear distinction among the levels of EPA in the brine shrimp, as the comparison between the CH and the other cyst sources demonstrate. Watanabe et al. (1983) reported that growth and survival of red sea bream improved when fed brine shrimp with high levels of EPA. Webster (1989) found significantly better growth and survival of striped bass larvae fed brine shrimp nauplii containing 10% (of the lipid) EPA than those fed nauplii containing less than 3% EPA. The high percentage of EPA

TABLE 2. *Hatching characteristics for four sources of brine shrimp cysts.*

| Source[1] | Hatching time[2] $t_{10}$ (h) | Hatching time[2] $t_{50}$ (h) | Hatching time[2] $t_{75}$ (h) | Total hatching percentage (% ± SE) | Hatching yield[3] (Number of nauplii × $10^5$ per g cysts ± SE) | Naupliar length (μm ± SE) |
|---|---|---|---|---|---|---|
| SFB | $12.1^{b}$ | $16.5^{b}$ | $21.0^{b}$ | $85.1 \pm 1.5^{a}$ | $1.62 \pm 0.2^{a}$ | $382 \pm 16^{c}$ |
| COL | $11.8^{a}$ | $13.4^{a}$ | $14.9^{a}$ | $85.9 \pm 0.7^{a}$ | $1.22 \pm 0.12^{ab}$ | $455 \pm 20^{b}$ |
| GSL | $12.7^{c}$ | $17.9^{c}$ | $25.2^{c}$ | $82.6 \pm 3.9^{a}$ | $1.12 \pm 0.03^{ab}$ | $485 \pm 9^{ab}$ |
| CH | $16.8^{d}$ | $25.6^{d}$ | — | $67.5 \pm 1.5^{b}$ | $0.87 \pm 0.12^{b}$ | $500 \pm 11^{a}$ |

[1] Sources of brine shrimp are San Francisco Bay (SFB), Colombian (COL), Great Salt Lake (GSL), and Chinese (CH).

[2] $t_{10}$, $t_{50}$ and $t_{75}$ is the time required for 10, 50, and 75% of the cysts to hatch. Values are predicted values from the nonlinear analysis. Values with different superscripts are significantly different ($P < 0.05$).

[3] Values represent means ± SE of 2 replications. Means with the same superscript are not significantly different ($P > 0.05$).

found in the CH brine shrimp suggests that this source might be nutritionally superior to the other sources for feeding larvae for an extended time period when other sources of nutrients are not available. Other sources of brine shrimp may be suitable for short term feeding, e.g., striped bass larvae grew and survived satisfactorily on low EPA brine shrimp for 8 days, but not 12 days or longer (Webster 1989).

Marked variation in hatching quality is also present among the sources of brine shrimp. Hatching quality may sometimes be more important than nutritional quality, especially if the brine shrimp are given for only a short time before other sources of nutrients become available. A slow hatching rate can create a problem in that the first-hatched nauplii may change from instar I to instar II by the time hatching is completed. The energy and lipid contents of instar II nauplii are reduced to 27% of instar I nauplii (Sorgeloos 1980). Hatching rate can be influenced by methods of harvesting and processing the cysts, storage time and conditions, and hatching environment (Vanhaecke and Sorgeloos 1982; Vos et al. 1984). Our hatching temperature was 27 C. Different hatching temperatures may have produced hatching rates different from those reported here (Vanhaecke and Sorgeloos 1983; Vos et al. 1984).

Hatching percentage and hatching rate are not the sole criteria for evaluating quality of brine shrimp cysts. The number of nauplii produced per g of cysts, or yield, is often an important consideration to hatchery personnel because a large number represents additional feeding opportunities for the larvae. The SFB source produced one-third more nauplii per g of cysts than the COL or GSL sources and nearly twice as many as the CH source. Production of one million nauplii would require 6.2, 8.1, 8.5, and 11.5 g of the SFB, COL, GSL, and CH cysts, respectively. Vanhaecke and Sorgeloos (1980) recognized that the number of nauplii per g of cysts may underestimate the weight of nauplii produced, especially from large cysts, and proposed biomass (weight) of nauplii produced per g of cysts as a useful criterion for hatching quality (Vanhaecke and Sorgeloos 1983). However, the length of time post-hatch when nauplii weight is measured is critical, especially when comparing fast and slow-hatching cysts, because chemical composition and body density of the naupliar change with age. Large naupliar size is undesirable for larval fish with small mouth gape (Smith 1976).

Although the CH cysts had the poorest hatching quality among all sources, they had the highest level of EPA. If the brine shrimp are given for only a short time or are supplemented with other nutritional sources, the other sources of cysts may be preferable because of faster and higher hatching rates. These results show that a great amount of variation occurs in hatching quality, naupliar size, and nutritional quality among commercial sources of brine shrimp cysts; all of these critera should be used to evaluate the quality of brine shrimp for larval feeding. It should be expressed that variation in nutritional and hatching quality occurs among batches of cysts from the same geographic origin, caused by changes in environment, strain of brine shrimp, and cyst processing procedures (Vanhaecke and Sorgeloos 1983).

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