ALGAE-FED BRINE SHRIMP NAUPLII AS A FOOD SOURCE FOR LARVAL SPOTTED SEATROUT

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Abstract: Larval spotted seatrout (*Cymoscion nebulosus*) grew faster on 1 and 2 day prefed brine shrimp (*Artemia salina*) nauplii than fish fed newly-hatched nauplii. Fish growth and survival were reduced when 3 and 4 day prefed, as compared with newly-hatched nauplii were offered. Proximate analysis and calorimetry demonstrated the occurrence of a temporal decline in the nutritional value of both fed and unfed brine shrimp nauplii. At the relatively low levels of algal cell concentration available for prefeeding brine shrimp, this study indicates that 1 day old unfed nauplii are nutritionally equal to algae prefed nauplii as a food source for larval spotted seatrout. One day old brine shrimp nauplii are a better food organism for larval spotted seatrout 12 to 21 days of age than newly-hatched nauplii because of the larger size of the former.

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One of the most critical challenges to closing the life cycle of marine fish in the laboratory is rearing them from eggs through the larval stage. The greatest problem is providing the larvae with adequate food, assuming they will not accept an artificial diet. Suitable food organisms must be easily procured or reared in the laboratory. The food organisms should be: (1) of the proper size, (2) continuously available, (3) present at the proper concentration, (4) of adequate nutritional value, and (5) available in progressively larger sizes as the larvae grow.

Newly-hatched nauplii of brine shrimp have been widely used as a food organism in rearing marine fish larvae. This study was undertaken to determine the advantages, if any, of prefeeding brine shrimp nauplii on algae prior to using them as food for larval sciaenids. Pertinent factors examined included the best duration and algal concentration for prefeeding brine shrimp nauplii in order to increase their nutritional value to larval sciaenids, and size differences among brine shrimp unfed and fed for different time periods. The study was conducted with larval spotted seatrout but the results should be applicable to other sciaenids.

Brine shrimp have been widely used for many years as food for fish larvae (May 1970). Sorgeloos (1976) noted that the use of brine shrimp nauplii in mariculture has greatly increased in recent years creating a demand far in excess of the present supply of cysts. This may create a limitation to future expansion of mariculture and indicates that research is needed to develop methods of obtaining optimum use from existing brine shrimp supplies.

In most studies newly-hatched brine shrimp nauplii have been used. Morris (1956) stated that it is best to feed newly-hatched nauplii to fish larvae, since the nauplii will be rich in nutritious yolk material. After hatching the nauplii grow rapidly and utilize their yolk supply, thus providing less energy and nutrients to larval fish. Sorgeloos (1975) substantiated this with proximate analysis and calorimetry, comparing newly-hatched with 1 day old nauplii. He reported a 22 percent decrease in organic substances and a 24 percent decrease in the caloric content within 24 hr. Morris (1956) related that in some cases fish larvae which were fed on older brine shrimp nauplii did not grow as well as those feeding on newly-hatched nauplii.

Brine shrimp nauplii which have been fed on algae may in some cases be more nutritious than newly-hatched nauplii. Rosenthal (1969) successfully reared herring larvae on brine shrimp nauplii that had been prefed on the algae *Dunaliella* sp. Morris (1956) found greater survival of herring larvae when the older nauplii were fed on *Dunaliella* sp. and *Stichococcus* sp. than when the nauplii were not fed. Blaxter (1962) found no significant difference between herring larvae fed newly-hatched nauplii and nauplii fed *Chlorella* sp. However, Sick (1976) found that *Chlorella conductrix* was not a good algae for brine shrimp growth because it was poorly digested by nauplii which expended large amounts of energy filtering this algae from the water. Arnold et al.

(1978) successfully reared spotted seatrout larvae using brine shrimp nauplii prefed on the alga *Tetraselmis chuii* starting when the fish larvae were 13 days old. The spotted seatrout larvae were initially fed rotifers and then newly-hatched brine shrimp nauplii. This procedure had good results but no comparative studies of larval fish growth or survival on prefed as opposed to newly-hatched brine shrimp nauplii have been conducted. None of the larval fish studies to date relate how long the nauplii were prefed on algae or what concentrations of algae were used in prefeeding.

MATERIALS AND METHODS

Eggs of spotted seatrout spawned at the Port Aransas NMFS Laboratory by the method of Arnold et al. (1978) were collected the morning following the night of spawning. The eggs were collected by dipping them out of the spawning tank filter box, where they were found floating at the surface, and placing them into a 100 ml beaker. In preliminary trials, the eggs contained in a 100 ml beaker were individually counted in small quantities in a petri dish with a dissecting microscope to determine the total number. Since this method damaged some eggs and lowered hatch rates, in the experiments the number of eggs stocked were approximated on the basis of the average number known to be contained in a 100 ml sample.

The flagellated spherical green alga *Tetraselmis chuii* was cultured for use in this study. The alga was cultured in duplicate fiberglass tanks 1.8 m in diameter and 76 cm deep. A water depth of approximately 65 cm resulted in each tank holding about 1,650 l of water. Each tank was equipped with airlift circulation tubes and 2 light strips 122 cm long holding 4 "cool white" 40 w fluorescent light bulbs, positioned about 60 cm above the surface of the water. Water for algal culture was pumped from the Corpus Christi Channel, flowed through a settling pond, and filtered through a double thickness of 80-grade Nytex screen or a 1 micron filter to remove contaminating organisms. The culture was started by adding filtered seawater to 100 l of algal stock in a concentration of about 20,000 cells/ml. An algal tank was fertilized by disoslving 500 g each of ammonium sulphate and superphosphate in the water. After about 2 days, 75 percent of the alga tank was harvested and then refilled with filtered seawater. Once a week an additional 250 g each of ammonium sulphate and superphosphate were added to refertilize the tank. The salinity of the algal culture was 28 $\pm$ 3 ppt and temperature was maintained at 26$\pm$ 2 C.

Cysts (20 to 40 g) of San Francisco Bay brine shrimp were placed in a 10 l glass carboy. The carboy was filled with filtered seawater (28 $\pm$ 2 ppt and 27 $\pm$ 1 C) to the bottom of the neck, fitted with an airstone to produce turbulent aeration, and allowed to incubate. Most cysts hatched and were harvested by about 24 hr under the conditions outlined. For harvesting the airstone was removed, the carboy was slighly inclined to one side, and allowed to sit in that position for about ten min. The unhatched cysts and empty shell cases floated to the top and the newly hatched nauplii settled to the bottom. This allowed the nauplii to be siphoned off the bottom into a cloth dipnet.

Initial algal concentrations in the feeding tanks ranged from 1,000 to 20,000 cells/ml. Feeding tanks were fertilized with 150 g each of ammonium sulphate and superphosphate when filled with algae culture water. The algae reproduced faster than the brine shrimp nauplii could consume the cells, therefore algal concentrations continued to rise during the brine shrimp nauplii feeding period. Final algal concentrations at the time of nauplii harvest ranged from 4,000 to 38,000 cells/ml. Algal cell concentrations were determined daily.

A number of samples of nauplii of the different prefeeding periods and unfed ones of the same age were collected. Among the samples of prefed nauplii were ones from different levels of algal concentrations. An initial subsample was analyzed for water percentage and the remainder frozen for later proximate analysis and calorimetry. Another group of nauplii samples (including newly-hatched, unfed for 1 and 2 days, and prefed for 1, 2, 3, and 4 days) was taken, in buffered 5 percent formalin, and measured to the nearest 0.01 mm under a dissecting microscope.

The larval spotted seatrout were reared in duplicate 75 l glass aquaria. Each aquarium was equipped with an airstone to provide gentle aeration and covered with a sheet of plexiglass. The aquaria were arranged in 2 banks, one above the other, supported by a wooden framework. Each bank had a row of 4 fluorescent lights with 40 w bulbs positioned 6 to 9 cm above the aquaria to provide constant illumination. Immediately before being stocked with eggs, the aquaria were filled with water which contained algae to act as a biofilter in the removal of metabolic wastes from the fish. For each experiment a total of 8 aquaria were each stocked with approximately 5,000 seatrout eggs.

Water temperature and salinity were measured daily in each aquarium. Salinity was 30 ± 3 ppt for each experiment. Temperatures started at 27 C (the same as the water the eggs were spawned in) and by the next day climbed to 31 ± 1 C for the upper bank of aquaria or 30 ± 1 C for the lower bank. The number of seatrout larvae remaining in each aquarium was estimated daily to establish percentage survival for the experimental period. A 1 ml sample of water was taken from each aquarium daily with a syringe and the number of food organisms in it was counted in a petri dish through a dissecting microscope. Adjustments were made when proportionally equal numbers of food organisms were not present in the various aquaria. The fish in each aquarium were fed daily at a rate which resulted in the presence of few food organisms after 24 hr. Approximately the same density of food organisms per larval fish was fed to all aquaria.

The larval seatrout began feeding on rotifers the second day after hatching. By day 5 some of the seatrout larvae were large enough to take newly-hatched brine shrimp nauplii. Newly-hatched nauplii were fed to all the seatrout larvae from days 5 through 11. Experimental treatments began on day 12 when half the seatrout aquaria were offered prefed nauplii and the other half of the aquaria continued on newly-hatched nauplii. In one experimental trial the nauplii were prefed on algae for 1 and 2 days before being fed to the seatrout larvae and in the other, the nauplii were prefed for 3 and 4 days. The experimental trials ran from day 12 through day 21. On day 21, all the seatrout larvae were removed from the aquaria and individually measured to the nearest 0.25 mm total length. The fish from each aquarium were retained for proximate analysis.

Percent water was determined directly by measuring the weight difference between wet and oven dried samples. Nitrogen was determined according to the method of Cobb, et al. (1973) using micro-Kjeldahl digegstion, Conway diffusion, and titration. Percentage lipid was determined by chloroform-methanol extraction. Percentage ash was determined directly by placing dried samples in a muffle furnace at 600 C overnight and measuring weight loss on ignition. Calorimetry was performed on dried brine shrimp nauplii samples using a Parr adiabatic bomb calorimeter.

Chi square ($P < .05$) was used to test for significant differences between treatments. One-way analysis of variance ($P < .05$) was employed to test for significant differences within treatments over time (Walpole 1968). When significant differences were found, a multiple range test (Kramer 1956) was used to delineate them.

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RESULTS AND DISCUSSION

The average growth and percent survival of the larval seatrout for the experimental periods are presented in Table 1. The results of individual aquaria were paired on the basis of the initial number of fish at the time the experimental period started for comparison of average total length and percent survival. This was done to standardize effects of density on growth rate and survival. Starting at 8 to 10 days of age, the larval seatrout became cannibalistic. This problem has been experienced in previous attempts to rear seatrout in the laboratory (Arnold et al. 1978). Cannibalism was more pronounced in aquaria housing larval seatrout which were fed with prefed nauplii. In 5 of the 8 pairs of aquaria, survival during the experimental period was better in seatrout fed newly-hatched nauplii than those fed prefed nauplii.

Three of the 4 pairs of aquaria in the experiment using 1 and 2 day prefed nauplii produced better growth of larval seatrout as compared with seatrout offered newly-hatched nauplii and in the fourth pair growth was about equal. However, the greatest average growth in 1 of these pairs was probably because there were only 2 large cannibalistic larval seatrout surviving in the aquarium receiving prefed nauplii. In 2 out of 4 pairs of aquaria, survival was greater for larval seatrout fed newly-hatched nauplii.

In the experiment using 3 and 4 day prefed nauplii, fish growth was better in 2 of the pairs when fed newly-hatched nauplii than when offered prefed nauplii. Survival was greater for fish offered prefed nauplii in 1 of the pairs but less in the other. In the

Table 1. Growth and survival of larval *Cynoscion nebulosus* on newly-hatched and algal-fed brine shrimp nauplii.

Fish fed Newly-hatched A. salina				*Fish fed* A. salina *for 1 and 2 days*			
No. Start	*No. End*	*% Surv.*[a]	*Tl*[b]	*No. Start*	*No. End*	*% Surv.*[a]	*Tl*[b]
500	61	12	12.3	50	10	20	17.4
60	47	78	17.4	40	22	55	21.6
25	15	60	21.1	10	8	80	21.8
25	11	44	16.7	25	2	8	25.6
Fish fed Newly-hatched A. salina				*Fish fed* A. salina *for 3 and 4 days*			
200	180	90	16.6	200	99	50	13.0
100	62	62	17.8	90	69	77	14.5
75	45	60	17.9	100	8	8	19.9
10	6	60	17.2	20	1	5	20.0

[a]Spotted seatrout larval survival in aquaria from age 12 to 21 days old.
[b]Tl is mean total length in mm of all surviving fish in an aquarium at 21 days.

other 2 pairs, the average growth figures were greater for fish receiving the prefed nauplii but again this may have been because of the presence of only large, cannibalistic fish.

Proximate analysis data on larval seatrout (Table 2) indicated no statistically significant differences for any measurement among the 4 groups of fish. Table 3 presents a summary of the available data on proximate analysis of fish in the genus *Cynoscion*. The data from the present study are from samples of whole larval fish while the values in the other studies are from tissue samples of mature fish. The larval fish were 3 to 8 percent higher in water and 5 to 6 percent lower in protein with ash and lipid comparable in all animals.

Table 2. *Cynoscion nubulosus* larvae proximate analysis.

Food variable	*TL (mm)*[a]	*% Moisture*	*% Crude Protein*[b]	*% Lipid*[b]	*% Ash*[b]
Offered nauplii prefed for one and two days	17.40	82.7	78.6	9.7	—
	21.55	80.9	60.7	8.3	15.5
	21.84	78.6	65.9	6.5	—
	25.64	—	63.5	—	—
Offered newly-hatched nauplii	12.30	80.8	74.0	9.2	12.0
	17.35	79.4	59.0	7.1	15.6
	21.08	81.6	63.0	10.6	16.6
	16.72	80.3	71.1	11.7	—
Offered nauplii prefed three and four days	12.95	83.6	61.7	10.4	14.8
	19.93	83.1	75.7	12.3	—
	20.00	—	74.1	—	—
	14.48	84.1	62.9	9.0	19.2
Offered newly-hatched nauplii	16.60	85.0	64.0	15.3	15.3
	17.78	83.0	70.6	11.8	11.0
	17.16	81.9	82.9	12.9	—
	17.84	83.1	72.8	11.7	16.9
Average		82.0	68.8	10.5	15.2

[a]TL is mean total length of all surviving fish in an aquarium.
[b]dry weight basis.

Table 3. Comparison of *Cynoscion* spp. proximate analysis data from the present study with published information.

Study	*Species*	*% Moisture*	*% Protein*	*% Ash*[a]	*% Lipid*[a]
Glover (1970)	*C. nebulosus*	78.8	18.2	—	1.24
McGeachin et al. (1977)	*C. nebulosus*	82.0	12.5	2.6	1.7
		(78.6-85.0)	(9.7-15.0	(2.1-3.4)	(1.4-2.3)
Thompson (1959)	*C. sp.*	74.3	17.5	3.09	5.0
		(73.1-76.5)	(17.0-18.1)	(2.56-3.79)	(3.3-6.3)
Sidwell et al. (1974)	*C. regalis*	76.6	18.7	1.19	3.2
		(74.6-79.6)	(15.7-20.0)	(1.1-1.3)	(1.4-4.3)

[a]wet weight basis

Prefeeding nauplii for 1 and 2 days before offering them to larval seatrout produced faster growth but did not alleviate the problem of cannibalism. From the standpoint of survival, it appeared that until the fish were at least 21 days old, newly-hatched nauplii were as good, and possibly better, as food for larval spotted seatrout than prefed nauplii.

Variations in brine shrimp nauplii size with age and feeding are presented in Table 4. There were no statistically significant differences in size between fed and unfed nauplii on any given day following hatching up to 4 days. By the first day after hatching the nauplii almost doubled in size. Unfed nauplii increased 37 percent in length and fed nauplii increased 43 percent. One way analysis of variance showed both unfed and fed nauplii to have significant variation and multiple range tests showed newly-hatched nauplii to be significantly smaller than 1 day and older. One day old nauplii were significantly different from 2 day old and older nauplii (which were not significantly different from each other), but the additonal increase at 2 days was 9 percent for unfed and 5 percent for fed nauplii. Feeding the older spotted seatrout larvae on significantly larger nauplii can therefore be accomplished either by prefeeding nauplii on algae for 1 day or merely allowing growth for 1 day from yolk material. Feeding from the first through the fourth day did not produce significantly larger food organisms.

Table 4. *Artemia salina* nauplii size (TL in mm[a])[b].

Age of nauplii	*Unfed*	*Fed*
Newly hatched	.38a	.38a
One day old	.60b	.67b
Two days old	.71c	.74c
Three days old		.76c
Four days old		.76c

[a]TL is mean total length.

[b]Values within each column which bear the same letter are not significantly different ($P < 0.05$).

Brine shrimp nauplii proximate analysis data are presented in Table 5 and Fig. 1. There were no statistically significant differences between unfed and prefed nauplii; however, significant temporal changes occurred in both unfed and prefed nauplii. Only the average dry weight protein percentage remained the same over time. The average percent moisture increased sharply in both unfed and fed nauplii between hatching and 1 day of age. For unfed nauplii, multiple range tests showed newly-hatched to be significantly different from 1 and 2 day old animals. For fed nauplii, multiple range tests showed newly-hatched and 1 day old nauplii were not significantly different, nor were nauplii between 1 and 4 days of age.

The average dry weight lipid percentage significantly decreased with time for both unfed and fed nauplii. For unfed nauplii, newly-hatched and 1 day old animals were not significantly different and 1 and 2 day old nauplii were not significantly different. In fed nauplii, newly-hatched and 1 day old organisms were significantly different. This indicated that the most pronounced change was between newly-hatched and 1 day old

Table 5. *Artemia salina* nauplii proximate analysis[a].

	% Moisture		% Protein		% Lipid		% Ash	
Age of nauplii	*unfed*	*fed*	*unfed*	*fed*	*unfed*	*fed*	*unfed*	*fed*
Newly-hatched	83.7a	83.7a	48.1	48.1	17.7a	17.7a	5.0	50.a
One day old	91.1b	92.1a	50.6	54.8	13.9ab	13.2ab	6.6	9.2ab
Two days old	90.8b	94.7b	46.3	50.7	10.5b	12.4b	9.9	9.6ab
Three days old		94.3b		54.2		9.6b		7.9ab
Four days old		93.2a		46.4		7.7b		12.0b

[a]Values within each column which bear the same letter are not significantly different ($P < 0.05$).

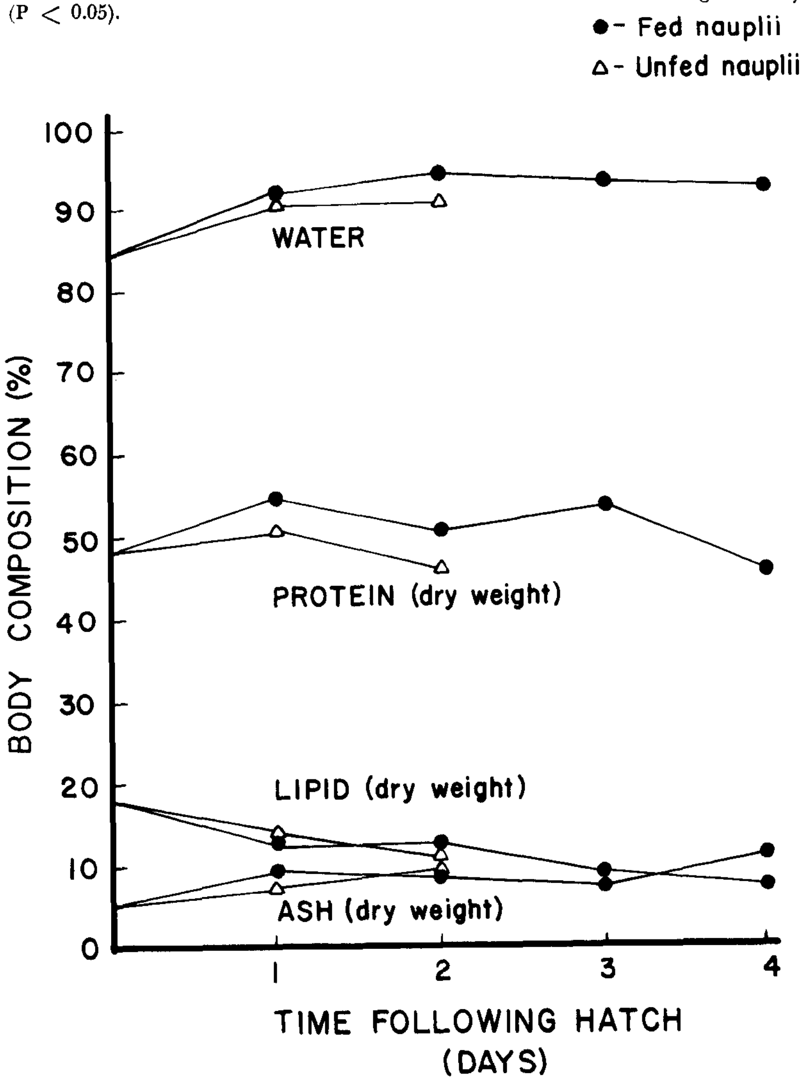

Fig. 1. Change in body composition with time of fed and unfed *Artemia salina* nauplii.

nauplii. This would be anticipated since the greatest growth in size was during that period. The decrease in lipids with time reflected their possible use by the nauplii as the primary source of energy. The decrease in lipids was visually corroborated as the nauplii were bright orange when newly-hatched because of carotenoid pigments associated with the yolk lipids. As the nauplii grew older they rapidly lost their orange color and turned transparent indicating the loss of yolk lipids (Morris 1956). Unfed nauplii were transparent by 2 days of age indicating the exhaustion of lipids and thus, their energy supply. Unfed nauplii died of starvation by 3 days of age. Fed nauplii similarly lost their orange coloration at the same rate, but at the end of the first day after hatching they had begun feeding on algae, and green algal cells were visible in the gut. The new energy supplied from algae may have gone to growth and metabolism because lipid levels continued to decrease indicating no excess energy available for lipid deposition.

Another possible explanation for the decrease in lipid percentage may be a reflection of growth with conservation of lipids already present. As the nauplii grow they may not be obtaining significant amounts of lipid from the algae and are retaining those present in the yolk and using them in cellular growth. As the nauplii grow, their bodies gain weight in water, ash and protein, but the absolute amount of lipids may remain the same while decreasing in terms of body percentage. The data and methods available to this study preclude determining which, if either, of these 2 possible explanations for the decrease in percent lipids applies.

Average percent ash increased with time for both unfed and fed nauplii. For fed nauplii the increase was statistically significant, and multiple range testing showed newly -hatched through 3 day old nauplii were not significantly different and 1 through 4 day old nauplii were not significantly different. The gradual increase in percent ash reflected the growing size and elongation of the naupliar exoskelton. Sorgeloos (1975) reported a 22 percent decrease in organic substances between newly-hatched and one day old San Francisco Bay nauplii. Although not statistically different, the percentage ash value for one day old unfed nauplii was less than that of 1 day old fed nauplii.

Table 6 presents the calories/g dry weight and the percent change with time for brine shrimp nauplii. There were no statistically significant differences between unfed and fed nauplii but the average values for fed nauplii were lower than were those of

Table 6. Average caloric values for *Artemia salina* nauplii.[a]

	Unfed		*Fed*	
	Cal/g dry wt.	*% Decrease*	*Cal/g dry wt.*	*% Decrease*
Newly-hatched	4933a	—	4933a	—
One day	4303b	12.8	4031b	18.3
Two days	3953c	19.9	3567c	26.7
Three days	—	—	3131d	36.6
Four days	—	—	3507cd	28.9

[a]Values within each column which bear the same letter are not significantly different ($P < 0.05$).

unfed nauplii. Sorgeloos (1975) reported a decrease in caloric content between newly-hatched and 1 day old nauplii of 24 percent. The present study indicated less decrease, only 13 percent for this time period, and the absolute values are different from those reported by Sorgeloos. Multiple range testing showed most of the values to be significantly different, with the exception of 2 to 4 day old fed nauplii and 3 and 4 day old fed nauplii which were not significantly different from each other.

The algae levels used in prefeeding brine shrimp nauplii were grouped into 3 ranges to compare results. These ranges were low (less than 10,000 algal cells/ml), medium (10,000 to 20,000 cells/ml), and high (from 20,000 to the maximum obtained in the cultures of 38,000 cells/ml). A comparison of the different levels in terms of average caloric values (calories/g dry weight) is presented in Table 7. There was a trend, though not statistically significant, for values to increase as algal cell concentration increased. Variations in brine shrimp nauplii lipid composition with respect to different concentrations of algal cells are presented in Fig 2. High algal concentration in prefeeding resulted in lipid levels close to those of newly hatched nauplii through the first 2 days of feeding.

Table 7. Comparison of nauplii caloric values in relation to algae levels used in pre-feeding.

Age of nauplii	*Low*	*Medium*	*High*
One day	3976	4067	—
Two days	3413	3456	3547

The results of brine shrimp nauplii proximate analysis and calorimetry support the results of spotted seatrout larval growth. Three to 4 day prefed nauplii were inferior to newly-hatched nauplii in terms of resulting seatrout growth as might be predicted by the differences between the percent lipid and caloric values of 3 and 4 day fed and newly-

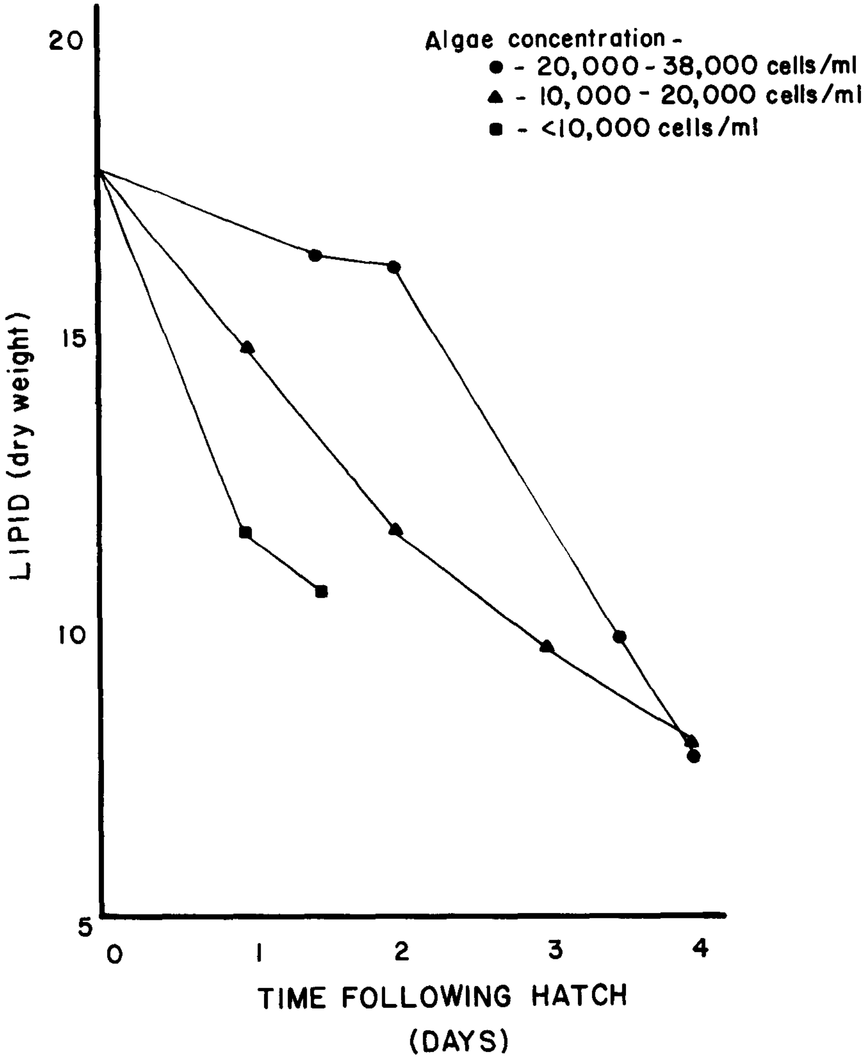

Fig. 2. Relationship between time following hatch of *Artemia salina* nauplii and dry weight lipid percentage with algal cell concentration.

hatched nauplii. The caloric and lipid values of 1 and 2 day old fed nauplii were lower but close to those of newly-hatched nauplii. The larger size of fed nauplii compared with newly-hatched animals would result in less energy being expended by fish to ingest an equal biomass of nauplii and could result in greater or equal growth of larval fish.

When viewed from a practical standpoint, since there was no significant difference in size, percent lipid or caloric value between 1 day old nauplii, fed or unfed, it would seem that the simplest technique for preparing food for older seatrout larvae would be to hold nauplii for 1 day after hatching in the hatching carboy. Since brine shrimp nauplii don't feed for most of the first day, little advantage can be obtained by providing them with algae immediately after they hatch.

CONCLUSIONS

1. A significant change occurs in brine shrimp nauplii between the time they are newly-hatched and 1 day old. The nauplii almost double in size, significantly increase in percentage water, and decrease in percentage lipids and caloric value during this time period.
2. Under the relatively low algae concentrations (less than 38,00 cells/ml) used in prefeeding the brine shrimp nauplii in this study, nauplii fed for 1 day were nutritionally no better than 1 day old unfed nauplii as food for 12 to 21 day old spotted seatrout larvae.
3. One day old brine shrimp nauplii (unfed of fed) are a better food for larval spotted seatrout 12 to 21 days old than newly-hatched nauplii.
4. Proximate analysis of larval spotted seatrout showed them to be 3 to 8 percent higher in water and 5 to 6 percent lower in protein than the adult fish.
5. The question of whether algae fed brine shrimp nauplii are more nutritious than unfed nauplii should be reexamined using a less cannibalistic species of larval sciaenid than the spotted seatrout and much higher concentrations of algae in prefeeding brine shrimp nauplii.

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