

مجله دانشکده دامپزشکی دانشگاه تهران دوره (۴۷) شماره (۱ و ۲) تهران (۱۳۷۱)

اثرات منابع چربی در غذا بر روی چربی و پروفیل اسیدهای چرب  
آرتمیای پارتنوژنیک و دو جنسی دریاچه ارومیه\*

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**خلاصه:** آرتمیای دریاچه ارومیه (*Artemia urmiana* (AU) یکی از منابع پر ارزش غذای زنده بمنظور توسعه پرورش آبزیان در ایران قلمداد میگردد. ولی مطالعات تغذیه‌ای روی تخم و لارو این آرتمیا (احمدی و همکاران ۱۹۹۰) نشان می‌دهد که بهره‌برداری مستقیم از منابع آن برای پرورش آبزیان آبهای شور مناسب نیست لذا پرورش متراکم آن با تغذیه از جلبکهای خاص یکی از راه‌حلهای جبران این نقیصه میباشد.

بنابراین نوزاد آرتمیای (Instar I-II) پارتنوژنز و دو جنسی دریاچه ارومیه را بلافاصله بعد از تولد به ظروف مجزائی منتقل و سپس با ۳ نوع غذا مورد تغذیه قرار دادیم. غذاها بترتیب عبارت بودند از: جلبک *Tetraselmis* که از نظر اسید چرب  $W_3:3:18$  غنی و از نظر اسید چرب  $W_3:5:20$  فقیر است، جلبک نامشخص دیگری که از حیث اسید چرب  $W_3:3:18$  فقیر و از نظر اسید چرب  $W_3:5:20$  غنی و بالاخره سبوس برنج که حاوی ترکیبات  $W_3$  نمیباشد بعد از تغذیه و رسیدن به بلوغ جنسی، چربی و پروفیل اسیدهای چرب آرتمیاهای پارتنوژنیک و دو جنسی را مورد سنجش قرار دادیم. این مطالعات نشان می‌دهند که:

۱- آرتمیای پارتنوژنیک ایران ۲-۳ روز زودتر از دو جنسی‌ها به بلوغ جنسی می‌رسند درحالتیکه غذای یکنواختی را دریافت کرده‌اند. آرتمیاهایی که از سبوس برنج استفاده نموده‌اند در ۲۵-۳۰ روزگی به مرحله بلوغ جنسی می‌رسند درحالتیکه آرتمیاهای غذا جلیبیکی ۱۸-۲۰ روز به بلوغ جنسی دست

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- می‌یابند. حداکثر تلفات در دوران رشد مربوط می‌شود به آرتمیای دوجنسی که از سبوس برنج استفاده کرده‌اند و حداقل تلفات متعلق به جلبک تتراسلمس بود.
- ۲- بطور عمومی اسیدهای چرب موجود در آرتمیا تابع اسیدهای چرب غذای مورد تغذیه‌اش می‌باشد.
- ۳- آرتمیای دریاچه ارومیه همانند سایر آرتمیایها قادر است که از اسیدهای چرب با زنجیره کوتاه اسیدهای چرب با زنجیره بلندتر را بسازد.
- ۴- بیشترین اسیدچرب در آرتمیای ارومیه  $1w9:18$  می‌باشد که با سایر آرتمیایهای دنیا مطابقت دارد.
- ۵- تفاوت‌های بین آرتمیای پارتنوژنیک و دوجنسی که غذای مشابه دریافت داشته‌اند از نظر پروفیل اسیدهای چرب زیاد نمی‌باشد.

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### Conclusion

The objective of this study was to obtain practical information on how different dietary fatty acid profiles effect the resulting fatty acid profile of two genotypically different *Artemia* fed similar diets. This study confirms the findings of Millamena et al. (1988) that the fatty acid profile of *Artemia* reflects the fatty acid profile of the diet. Genotypic expression did not account for nutritionally important differences.

The results show that a diet having a fatty acid profile which is well balanced in the fatty acids commonly found in *Artemia* (Leger et al., 1986) resulted in *Artemia* which have a normal fatty acid distribution as described by Leger et al. (1986). The major saturated and monoene fatty acids (16:0, 16:1 and 18:1w9) comprise 40 -60% of the total fatty acids found in *Artemia*. The major diene, 18:2w6, usually contributes less than 10%. The major w3 fatty acids are 18:3w3 and 20:5w3. Usually 18:3w3 is very abundant or very scarce (Leger et al. 1986). The level of 20:5w3 is usually inversely related to the level of 18:3w3. Tetraselmis has significant levels of both of these fatty acids which is reflected in the AU which fed on this diet. The AU fed a diet of Tetraselmis had a balanced fatty acid profile which was similar to that reported by Leger et al. (1986).

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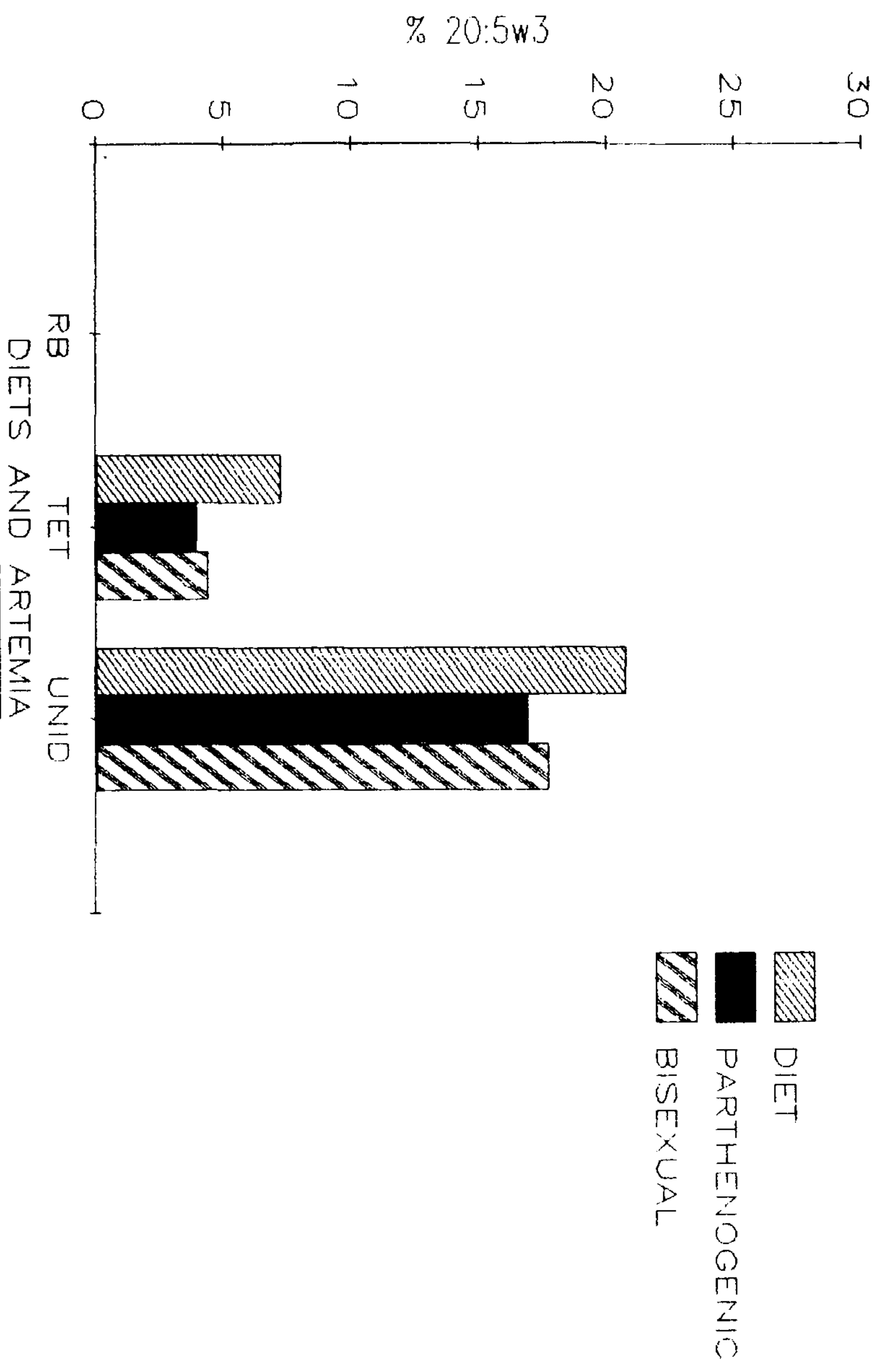
diet. The parthenogenic AU fed the unidentified algae showed higher levels of 18:2w6 and 20:2w9 than did the bisexual AU fed the same diet. Bisexual AU fed the unidentified algae had a higher level of 18:1w9 than the parthenogenic counterparts.

Tetraselmis is probably undergoes saturation to make up some of the 18:1w9 (29% parthenogenic and 34.53% bisexual) found in the AU fed Tetraselmis as the dietary level of 18:1w9 was only 6.38%. The additional 18:1w9 may have come from the elongation of shorter fatty acids to 18:0 followed by desaturation to 18:1w9. This can also explain similarly high levels of 18:1w9 (11% parthenogenic and 16% bisexual) in the AU fed the unidentified algae compared to the dietary level of only 2.5%. The elongation of shorter chain fatty acids to 18:0 or the de novo synthesis of this fatty acid may explain why the levels in the AU were higher than in the unidentified algae which they were fed.

$\beta$ -oxidation of some polyunsaturated fatty acids may explain why there is less of the fatty acid in the AU than in the diet. For example 18:4w3 is abundant in Tetraselmis (11%) yet there is no 18:4w3 in the AU fed this diet.

Differences between the fatty acid distribution of bisexuals versus parthenogenics were few. The bisexuals fed rice bran had 10% less 18:1w9 than the dietary level as compared to the parthenogenic AU fed rice bran which had similar levels. This observation is complicated by the higher lipid level (9.6%) found in the rice bran fed bisexual AU as compared to the rice bran parthenogenic AU (2.5%). The bisexual AU fed Tetraselmis show a higher level of 22:1 (2.33%) than the parthenogenic AU fed this

FIGURE 3. LEVELS OF 20:5w3 IN PARTHENOGENIC AND BISEXUAL UROMIAH ARTEMIA FED RICE BRAN, TETRASELMIS AND AN UNIDENTIFIED ALGAE COMPARED TO THE LEVELS IN THE DIETS.





acid distributions. Figure 3 shows the level of 20:5w3 found in the distributions of fatty acid in AU is dependent on the levels found in the diet. The higher the level of 20:5w3 in the diet the higher the level found in the AU.

Differences between the fatty acid distribution of the diets and of the AU fed the diets were seen. In order to determine the fate of the fatty acids consumed by the AU, radioactively labelled diets would have to be prepared. Explanations as to the differences between the distributions of fatty acids in the AU and their diets as well as between parthenogenic and bisexual AU fed the same diets are speculative.

An examination of the saturated fatty acids and monoenes of the AU shows that the corresponding levels in the diet may represent an increase or a decrease. AU fed rice bran showed an increase in the amount of 16:1w7 from a dietary level of 1.4% of the fatty acids to about 8% in the parthenogenic and bisexual AU. The percent of 18:0 was higher in the rice bran fed AU than in the rice bran. The percent of 16:0 and 18:0 was higher in the Tetraselmis fed AU than in the diet as was the percent of 18:0 in the AU fed the unidentified algae. These observations are probably explained by the ability of fish and probably Artemia to elongate a fatty acids from 12:0 to 16:0 and 18:0 or by de novo synthesis (Henderson and Sargent, 1985). The Artemia are able to desaturate 16:0 to 16:1 and 14:0 to 14:1. The 18:2w9 (16.47%) in the

FIGURE 2. LEVELS OF 18:3w3 IN PARTHENOGENIC AND BISEXUAL UROMIAH ARTEMIA FED RICE BRAN, TETRASELMIS AND AN UNIDENTIFIED ALGAE COMPARED TO THE LEVELS IN THE DIETS.

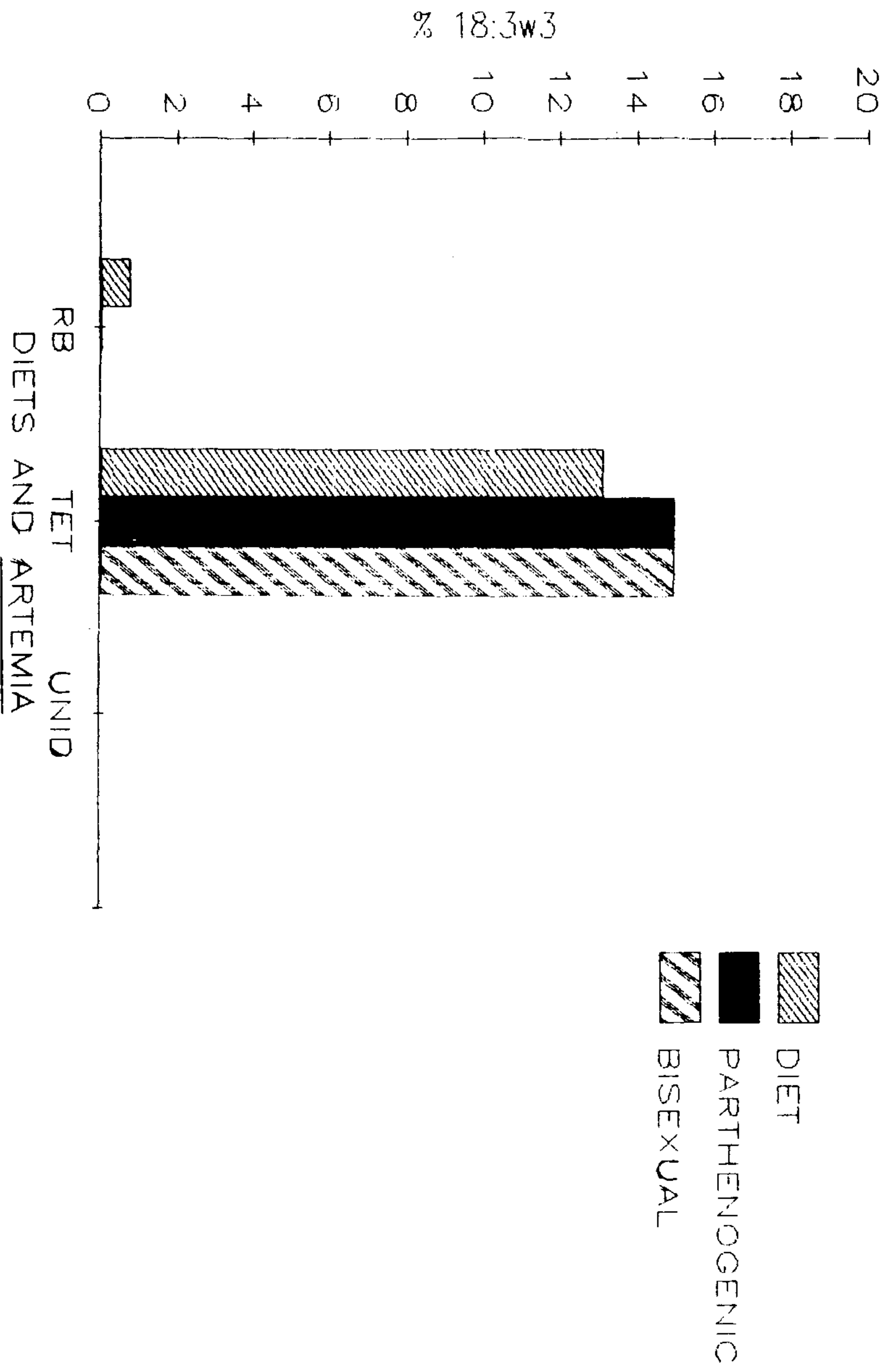
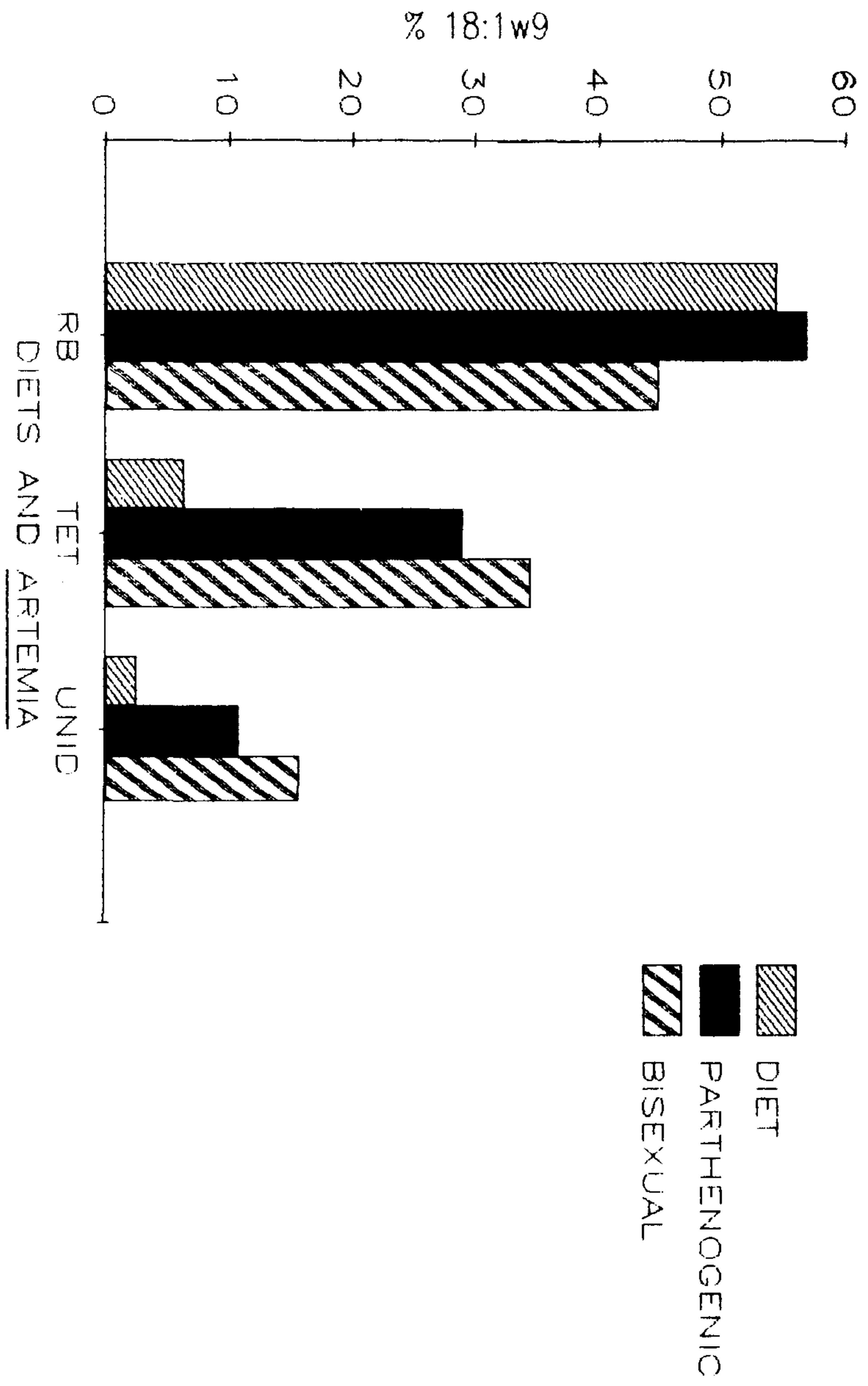


FIGURE 1. LEVELS OF 18:1w9 IN PARTHENOGENIC AND BISEXUAL UROMIAH ARTEMIA FED RICE BRAN, TETRASELMIS AND AN UNIDENTIFIED ALGAE COMPARED TO THE LEVELS IN THE DIETS.



in parthenogenic and bisexual AU fed rice bran, Tetraselmis and an unidentified algae to the level of 18:1w9 in the diets. In each case the level of 18:1w9 increases with the amount in the diet. Leger et al. (1986) in a review of all Artemia fatty acid distributions found 18:1w9 to be the most abundant. The levels of this fatty acid observed in the AU parthenogenic and bisexuals fed Tetraselmis are close to the mean range found by Leger et al. (1986) in other Artemia. Artemia must need higher levels of 18:1w9 as evidenced by the high level of 18:1w9 in AU fed a diet with a low level of the fatty acid and the observations of Leger et al. (1986). Those AU receiving the rice bran diet which contained very high levels of 18:1w9 had similarly high levels of this fatty acid. The bisexual AU were found to have 10% less than the distribution of rice bran however this level was also considered high. Millamena et al. (1988) showed that Artemia generally reflect the fatty acid distribution of the diet they eat. This fact explains why the level of 18:1w9 decreases with a decrease in the diet (fig 1). An examination of the level of 18:3w3 in the diets and the AU groups supports the findings of Millamena et al. (1988). Figure 2 shows the level of 18:3w3 in AU is dependent on the level in the diet. Only the AU fed Tetraselmis which has about 13% of 18:3w3 have 18:3w3 (about 15% in both parthenogenic and bisexuals). The other AU groups received little or no 18:3w3 in their diets and resulted in no 18:3w3 in their fatty

Table 4. Fatty acid distributions in parthenogenic and bisexual Uromiah lake artemia fed three experimental diets

Fatty acid	Parth RB (%)	Bisex RB (%)	Parth TET (%)	Bisex TET (%)	Parth UNID (%)	Bisex UNID (%)
12:0	0.11					
13:0						
14:0	2.02	5.50	1.97	1.15	4.36	3.33
14:1	1.87	1.36		0.37		0.78
15:0		0.39	0.16	0.34		
15:1	0.16	0.19				
16:0	19.11	29.07	23.03	18.63	12.11	13.41
16:1	8.30	7.92	5.44	4.30	30.87	33.19
17:0					1.82	1.36
16:2	0.10				1.60	1.46
18:0	5.59	5.32	8.51	7.60	10.72	8.43
18:1w9	56.85	44.85	28.99	34.53	10.92	15.76
18:2w9						
18:2w6	5.10	1.96	5.73	6.51	6.65	2.82
20:0			0.70	0.55		
18:3w3	0.06		14.94	14.94		
20:1	0.11	1.03				
18:4						
20:2			5.58	4.34	2.26	
20:3w3						
20:3w6	0.61	0.58			1.78	1.74
22:1			0.99	2.33		
20:5w3			3.96	4.41	16.93	17.73
24:1						
Unknown						

RB= Rice bran TET= Tetraselmis UNID = Unidentified algae

Table 4 shows the fatty acid distributions in parthenogenic and bisexual AU fed the experimental diets. The levels of 16:0, 16:1, 18:1w9, 18:2w6 and 18:3w3 in the parthenogenic and bisexual AU fed rice bran were 19.11% and 29.07% ,8.30% and 7.92, 56.85% and 44.85%, 5.10% and 1.96% and 0.06% and 0% respectively. No 20:5w3 was found in either AU groups fed rice bran. The levels of 16:0, 16:1, 18:0,18:1w9, 18:2w6,18:3w3 and 20:5w3 in parthenogenic and bisexual AU fed the *Tetraselmis* diet were 23.03% and 18.63%, 5.44% and 4.30%, 8.51% and 7.60%, 28.99% and 34.53%, 5.73% and 6.51%, 14.94% and 14.94% and 3.96% and 4.41% respectively. The levels of 16:0, 16:1, 18:0,18:1w9,18:2w6,and 20:5w3 in parthenogenic and bisexual AU fed the unidentified algae were 12.11% and 13.41%, 30.87% and 33.19%, 10.72% and 8.43%, 10.92% and 15.76%,6.65% and 2.82% and 16.93 and 17.73% respectively. No 18:3w3 was detected in either parthenogenic or bisexual AU fed the unidentified algae.

The three diets represent three distinctly different fatty acid distributions. Rice bran which is devoid of 20:5w3, had less than 1% of 18:3w3 and more than 50% of 18:1w9 in its distribution. By comparison, the unidentified algae has a high level of 20:5w3(20.76%), a low level of 18:1w9(2.57%) and no 18:3w3 in its distribution. In contrast, *Tetraselmis* has more of a balanced distribution having 6.38% of 18:1w9, 18.11% of 18:3w3 and 7.27% of 20:5w3.

Figure 1. shows a comparison of the level of 18:1w9

Table 3. Fatty acid distributions in rice bran, Tetraselmis and unidentified algae species

Fatty acid	Rice bran (%)	<u>Tetraselmis</u> (%)	Unidentified (%)
12:0	0.09	6.30	2.68
13:0	0.21	1.75	
14:0	0.83	0.38	6.02
14:1		0.34	
15:0	0.11	1.25	0.29
15:1		2.77	0.63
16:0	25.71	17.20	13.42
16:1	1.36	4.80	35.14
17:0			1.50
16:2		1.13	2.00
18:0	2.36	1.62	
18:1w9	54.45	6.38	2.57
18:2w9		16.47	
18:2w6	4.13	5.49	2.10
20:0		2.44	0.48
18:3w3	0.80	13.11	
20:1	2.14		
18:4w3	2.12	10.95	0.25
20:2	3.50		
20:3w3	0.10		0.67
20:3w6			2.56
22:1		0.99	
20:5w3		7.27	20.76
24:1			0.57
Unknown	2.10	0.17	

Table 2. Percent total lipids in parthenogenic and bisexual Uromiah lake Artemia and in the Tetraselmis, unidentified algae, and rice bran diets.

Diet	Reproductive Mode	Total lipid(%)
Tetraselmis	-----	4.9
Tetraselmis	Parthenogenic	1.9
Tetraselmis	bisexual	0.9
Unidentified	-----	5.1
Unidentified	Parthenogenic	2.3
Unidentified	bisexual	1.9
Rice bran	-----	8.6
Rice bran	parthenogenic	2.5
Rice bran	bisexual	5.9



Table 2. shows a comparison between the level of total lipids in the Tetraselmis, rice bran and the unidentified algae and the percent of total lipids on a wet weight basis in approximately one gram of pooled Artemia for each group of bisexual and parthenogenic AU fed each of the three experimental diets. Tetraselmis, rice bran and the unidentified algae had 4.9%, 8.6% and 5.1% total lipids respectively. Rice bran produced a higher level of total lipids in the bisexual AU (5.9%) than in the parthenogenic AU (2.5%). The levels of total lipids in the parthenogenic AU fed Tetraselmis and unidentified algae were 1.9% and 2.3% respectively as compared to the levels of total lipids in the bisexual AU fed these diets which were 1.0% and 1.9% respectively.

Table 3 shows the fatty acid distribution in Tetraselmis, rice bran and the unidentified algae. All of the three diets have a unique fatty acid profile which differentiates one from the other. Rice bran has 25.71% of 16:0, 54.45% of 18:1w9, 4.13% of 18:2w6, 0.8% of 18:3w3 and no 20:5w3. Tetraselmis has a more balanced distribution having 17.2% of 16:0, 6.38% of 18:1w9, 16.47% of 18:2w9, 5.49% of 18:2w6, 13.11% of 18:3w3, 10.95% of 18:4w3 and 7.27% of 20:5w3. The unidentified algae has 13.42% of 16:0, 35.14% of 16:1, 2.57% of 18:1w9, 2.10% of 18:2w6 and 20.76% of 20:5w3. The levels of other fatty acids in the distributions of the diets are shown in table 3.

Table 1. Final length(mm) of Uromiah lake Artemia fed three experimental diets until sexual maturity

Diet	Parthenogenic	Bisexual	
		Males	Females
Tetraselmis	8.3 3±0.6 2	8.8 9±0.6 7	10.5 0±0.8 4
Rice bran	8.0 0±0.6 0	7.5 8±0.6 36	9.0 7±0.9 8
Unidentified	7.8 5±0.3 5	7.4 1±0.4 9	8.2 4±0.7 6

in table 1. The lengths between parthenogenic and bisexual AU females did not vary significantly. The final lengths of the bisexual females were significantly longer than the bisexual males. The mean lengths of the parthenogenic, bisexual females and males fed Tetraselmis were 8.33 mm, 8.89 mm and 10.5 mm respectively. Those fed rice bran were 8.00 mm, 7.58 mm and 9.07 mm, respectively and those fed the unidentified algae reached 7.85 mm, 7.41 mm and 8.24 mm, respectively.

## RESULTS AND DISCUSSION

As individual premature female *Artemia* from the original cyst collection matured, their mode of reproduction became apparent when larvae began to appear in the beakers of those which were parthenogenic. The lack of larval production by the other females indicated that these were bisexual. This was the first sign of genotypic differentiation in the study. The larvae produced and subsequently fed *Tetraselmis*, unidentified algae and rice bran diets showed varying rates of development. Parthenogenic AU developed to sexual maturity 2 to 3 days sooner than bisexuals fed the same diets, however the diets also produce differences in growth rate. Parthenogenic AU fed *Tetraselmis* matured in 15 days as bisexuals took 18 days to reach sexual maturity. Parthenogenic and bisexual AU fed rice bran reached sexual maturity in 25 and 30 days, where those fed the unidentified algae reached sexual maturity in 18 and 20 days, respectively. Although no records were kept, the highest mortality was observed in the bisexual AU fed rice bran during the grow out period. The AU fed *Tetraselmis* appeared to have the least mortality.

The final length of the AU in this study is reported

The Tetraselmis and the unidentified algae were supplied by the U.S. Environmental Protection Agency Narragansett Laboratory. Rice bran suspension was prepared soaking and blending rice bran in filtered seawater followed by sieving the suspension through a 50 $\mu$  sieve. The solution was kept in the refrigerator and used for one week. Cultures were fed once a day and the water was changed every 3-7 days according to the water condition. After sexual maturation was completed, the total lengths of Artemia were measured. The Artemia were thoroughly washed with deionized water before extracting the lipids from pooled Artemia from each group amounting to about one gram of tissue. Lipid extractions were made according to the methods of Bligh and Dyer (1959) as modified by Kates (1972). Analysis of fatty acids by methylation followed by gas chromatography were made according to the methods of Schauer et al. (1980).

## MATERIAL AND METHODS

The Iranian brine shrimp(AU) cysts were collected from the western shoreline of Uromiah lake during the month of August,1987. The floating Artemia cysts(including algae and debris) were collected, cleaned, dried and kept in an airtight glass container under nitrogen in refrigerator until used. the cysts hatched in filtered sea water and the nauplii were fed the live algae (Tetraselmis or Isocrysis). For the analysis of the reproductive mode, premature female Artemia were separated and placed individually into one liter beakers containing aerated filtered sea water and algae for food. When sexually mature, the parthenogenic AU produced new larvae and the bisexual AU did not. After 10-14 days, 1-2 males were added to the original beakers containing the bisexual females. Once the males were placed with the females larvae were soon produced and they developed into males and females. The parthenogenic larvae developed into all females. Care was taken to prevent cross contamination of the pure cultures parthenogenic and bisexual AU established in the laboratory.

Newly hatched Artemia(instar I and II) from each of the pure cultures of parthenogenic and bisexual AU were transferred to new 3000 ml jars containing filtered sea water and aerated continuously. All culture containers were isolated from one another. Three containers of parthenogenic and three of bisexual AU received one each of either Tetraselmis, an unidentified algae or rice bran.

The purpose of this study, therefore was to culture AU on the following diets with the theoretical fatty acid compositions in parentheses: a) *T. suecica* (low levels of 20:5w3, high levels of 18:3w3), b) unidentified algae (high levels of 20:5w3, low levels of 18:3w3), c) rice bran lack of w3 fatty acids, high levels of w6 and w9 fatty acids). When the AU reached adulthood, analysis of their fatty acid profiles would provide clues to the ability of AU to utilize or transform the fatty acids available in the three different diets. The fatty acid profiles of the diets would also be analyzed to verify the theoretical composition in the experimental design.

## Introduction

The brine shrimp from lake Uromiah (*Artemia urmiana*) represent a potentially useful food source for aquaculture in Iran (Takami, 1987). An analysis of the nutritional composition of Uromiah Lake Artemia (AU) cysts and nauplii, however, indicates that the natural population of brine shrimp collected from the lake may be inadequate as a food for cultured marine organisms (Ahmadi et al. 1990).

Artemia of better nutritional quality might be obtained by intensive culturing of local AU fed with cultured algae or rice bran.

Preliminary feeding trials indicated that AU grew to adulthood more quickly and with better survival when they were fed the flagellates *Tetraselmis suecica* than, unidentified algae or rice bran. Both *T. suecica* and the unidentified algae apparently have substantially higher levels of the essential fatty acid for marine organisms (20:5w3) than does rice bran (Sorgeloos, personal communication). Rice bran lacks w3 fatty acids, but is rich in w6 and w9 series fatty acids (food composition tables). Schauer and Simpson (1985) showed that the bioconversion levels of Artemia are very low. I felt that an experiment in which AU were fed diets with different fatty acid profiles, in conjunction with fatty acid analyses of the diets and the resulting adult Artemia, could provide useful practical information on the quality of the different foods for intensive culture of AU.



C) Rice bran(lack of w3 fatty acids).

After feeding and when the AU(Parthenogenic and Bisexual)reached adulthood, the total lipids and fatty acid profile of both culture were analyzed. This study showed that:

- 1) Parthenogenic AU reached to sexual maturity 2 to 3 days sooner than bisexuals fed the same diets.Parthenogenic AU fed Tetraselmis matured in 15 days and bisexuals took 18 days to reach sexual maturity.Parthenogenic and bisexual AU fed rice bran reached sexual maturity in 25 and 30 days, where those fed the unidentified algae reached sexual maturity in 18 and 20 days, respectively.The highest mortality was observed in the bisexual AU fed rice bran during the grow out period.The AU fed Tetraselmis appeared to have the least mortality.
- 2) Iranian brine shrimp,generally reflects the different types of fatty acids contained in their diets.
- 3) AU are able to elongate the shorter-chain-fatty-acid to longer-chain-fatty-acids.
- 4) Fatty acid 18:1w9 seems to be the most abundant in AU and is similar to the other Artemia in the world.
- 5) Only a few differences between the fatty acid distribution of bisexuals versus parthenogenics which fed the same diet, were observed.

Effect of dietary lipid source on the  
lipid and fatty acid profiles of par-  
thenogenic versus bisexual Uromiah  
lake Artemia

\* \*  
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**Abstract:**

One of the most important live food source for developing aquaculture in Iran is the Iranian brine shrimp "Artemia urmiana"(AU). But the latest nutritional study on its cysts and nauplii have showed (Ahmadi et al. 1990), that the natural population of brine shrimp collected from the Uromiah lake may be inadequate as a food for cultured marine organisms. Therefore a better nutritional quality might be obtained by intensive culturing of AU fed with cultured algae.

However, the newly hatched parthenogenic and Bisexual AU (instar I-II) were transferred separately to the different jars and then feed them with the three experimental diets with the theoretical fatty acid composition as follows:

- a) Algae Tetraselmis (low levels of 20:5w<sub>3</sub>, high levels of 18:3w<sub>3</sub>),
- b) Unidentified algae (high levels in 20:5w<sub>3</sub>, low levels of 18:3w<sub>3</sub>)

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