

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorpjn@gmail.com

## Development of Salinity Stress Tests for Larval Striped Bass, *Morone saxatilis* and Inland Silver Sides, *Menidia beryllina*, Used in Nutritional Studies

Muhammad Ashraf<sup>1</sup>, David A. Bengtson<sup>2</sup> and K.L. Simpson<sup>3</sup>

<sup>1</sup>Department of Fisheries and Aquaculture,

University of Veterinary and Animal Sciences, Outfall Road, Lahore, Pakistan

<sup>2,3</sup>Department of Food Science and Nutrition, University of Rhode Island, Kingston, R.I. 02881, USA

**Abstract:** Salinity "stress tests" have previously been developed to detect subtle differences in physiological condition of larval fish between treatment groups in nutritional studies, when no differences exist in survival and growth. Methods that have been described in literature for Asian species include a) netting the fish out of water for a few seconds and measuring recovery time after its re-immersion and b) subjecting larvae to salinity stress for 2 h and measuring differences in mortality. In this study, we attempted to define the conditions for 15-h salinity stress tests with larval striped bass and 2-h salinity stress tests with inland Silver sides where a sharp delineation exists between survival and mortality and finally to use this test in a study of fatty acid requirements of the Silver sides. Striped bass larvae were cultured in an aquarium containing 5‰ water and fed on Reference *Artemia* nauplii for two weeks. Silverside larvae were reared in 30‰ sea water and fed on fatty acid enriched or un-enriched Great Salt Lake *Artemia* nauplii for three weeks. Striped bass were then exposed to 10, 20, 30 and 40 ‰ salinities while inland Silver sides to 40, 60, 65, 70, 75, 80 and 100‰ salinities and their survival was determined at ½ h intervals. Striped bass exhibited maximum survival at 20‰ and below. About 15% died in 30‰ water while all were dead in 40‰ at the end of 2-h period. Mortality count was increased when exposure time was prolonged. Fish larvae displayed significant ( $p < 0.05$ ) differences in mortality when duration between feeding and their exposure to stress was increased. Silverside larvae displayed maximum survival at 60‰ salinity and below but those raised on fatty acid enriched nauplii performed significantly ( $p < 0.05$ ) better than the controls in various stress tests.

**Key words:** Striped bass, inland silverside, salinity stress tests

### INTRODUCTION

Brine shrimp (*Artemia*) nauplii are used as a convenient source of food for larval rearing of fish and crustaceans. These nauplii are hatched from dormant cysts which are collected from various places all over the world. As the quality of these cysts is very much dependent upon the environmental factors, their nutritional value varies from source to source (Leger *et al.*, 1986). This variability in nutritional quality of *Artemia* nauplii can affect the physical condition of the larvae (Dhert *et al.*, 1990). Therefore, larval survival, growth and tolerance to unfavourable environmental factors in the culture ponds, is very much dependent on *Artemia* quality (Kuhlmann *et al.*, 1981; Leger *et al.*, 1986; Watanabe *et al.*, 1978, 1980; Kontara *et al.*, 1995). This persistent variation in the quality of the larvae and their unpredictable future has led the aquaculturists to assess the quality of the larvae, before introduction into the ponds; one of the prerequisite for a sustained growth of aquaculture industry. Only quality seeds can guarantee high growth and resistance to stress when exposed to unfriendly environment (Santhanakrishnan and Visvakumar, 1995).

Larvae therefore, are examined for colour, activity and muscle development (Tackaert *et al.*, 1989). Salinity stress tests are commonly applied in shrimp hatcheries to estimate the quality of Post Larvae (PL) to be used during grow out (Placios *et al.*, 2004). Stress tests in which larvae are netted from an aquarium and exposed to air for a few seconds and then returned to the water (Watanabe *et al.*, 1982) are difficult to standardize and analyze statistically. In recent years the problem of finding a simple procedure to evaluate the quality of post larvae produced in commercial hatcheries has led to the development of stress tests, most of them using salinity (Tackaert *et al.*, 1989; Rees *et al.*, 1994) or pH shocks (Arellano, 1990) or formalin shocks (Bauman and Jamandre, 1990). Recently attempts have been made to standardize stress tests for fish larvae by exposing them to very high salinity for 2 h, determining mortality and assessing physical state of the larvae (Dhert *et al.*, 1990). The development of similar methods for other species cultured in the various parts of the world might be useful not only for research purposes such as evaluation of diet effectiveness, but also as a

simple tool for the farmer to assess the quality of the fry before stocking.

Hence it was decided to develop a stress test in which striped bass (*Morone saxatilis*) and inland silverside (*Menidia beryllina*) larvae were subjected to salinity shock. Survival was observed on fed and unfed state in the former while in the later response of fish was determined when fed on enriched and un-enriched *Artemia* nauplii. Survival percentage of the larvae after 2 h period was assumed to be an indicator of their physiological state and nutritional effectiveness of the diet provided during rearing.

### MATERIALS AND METHODS

**Holding conditions:** Striped bass larvae obtained from the University of Maryland's Crane Aquaculture Facility, were transferred to a 75-L aquarium and acclimated for three days. Four hundred and fifty fish (11 d old) were randomly transferred from that aquarium into an adjacent 75-L aquarium (5 ‰) for experimentation. One hundred fifty larvae were equally divided into three 75-L (2 larvae L<sup>-1</sup>) aquaria and fed on Reference *Artemia* II nauplii (RAC II) (Bengtson *et al.*, 1985) for two weeks. Every day 4 g of dry *Artemia* cysts were placed in a 500 ml separatory funnel. They were incubated in filtered sea water (25±1°C) for 36 h under continuous aeration and constant light. After 36 h *Artemia* nauplii were harvested on a 150 µm sieve, rinsed with clean sea water and fed to larval fish.

Three hundred silverside larvae (10 d old), hatched from a single batch of eggs, spawned in laboratory were equally divided into 2 75-L (2 larvae L<sup>-1</sup>) aquaria. Silver sides in one aquarium served as control and were

fed on 48-h-old Great Salt Lake *Artemia* nauplii. The second group was offered *Artemia* nauplii from the same batch of cysts after enrichment with a commercial HUFA-enrichment product (Selco, *Artemia* Systems, Ghent, Belgium) hereafter referred to as *Artemia* Enrichment Product (AEP). These nauplii contained higher HUFA concentrations (Table 1). Group 1 was fed un-enriched Great Salt Lake *Artemia* while 2<sup>nd</sup> on HUFA enriched nauplii for three weeks.

Every day 3 g of Great Salt Lake cysts were put in two separatory funnels containing filtered sea water (30 ‰). The cysts were incubated for 29 h under constant fluorescent light and vigorous aeration. After 29 h, aeration in one separatory funnel, was stopped, *Artemia* nauplii were harvested and rinsed thoroughly with distilled water. Pre-weighed 1.2 g AEP was poured into a 2-L beaker containing 1 liter filtered sea water and homogeneously mixed. The nauplii were then transferred to the freshly prepared AEP solution, which was then poured into clean 2-L separatory funnel. The aeration was reinstated. After 19 h nauplii from both funnels (enriched + un-enriched), were harvested, rinsed with de-ionized water and fed to the respective treatment groups.

Both fish species received an *ad libitum* diet of live *Artemia* nauplii once a day, seven days a week. The fish in the aquaria received ambient photoperiod and continuous aeration. Every morning, before feeding, the aquaria were cleaned. Uneaten food, dead *Artemia* nauplii, cysts, any dead fish and accumulated debris, were siphoned out. Every day ¼th of the total aquarium water was removed and replaced by new sea water of the appropriate salinity.

Table 1: Fatty acid composition of reference *Artemia* cysts II (RAC II), great salt lake *Artemia* (GSL), *Artemia* enrichment product; selco (AEP) and AEP-enriched GSL *Artemia* nauplii (values are in mg fatty acid/100 mgs of total fatty acylmethylesters (FAME))

FAME	RAC II nauplii	GSL <i>Artemia</i> nauplii	AEP	AEP enriched GSL <i>Artemia</i> nauplii	Level of incorporation
13:0 (Tridecanoic)	-	10.0±0.9	-	-	-
14:0 (Tetradecanoic)	1.2±0.1	6.5±0.6	6.7± 0.3	2.0± 0.1	-4.5
15:0 (Pentadecanoic)	0.7±0.0	0.5±0.0	0.4± 0.0	0.8± 0.0	+0.3
15:1	0.5±0.1	-	ND	0.3± 0.0	-
16:0 (Palmitic)	13.7± 1.0	12.8±0.0	15.6± 1.3	11.0± 0.7	-1.8
17:0 (Margaric)	3.2± 0.0	1.0±0.0	ND	6.3± 0.1	+5.3
16:1ω7 (Palmitoleic)	13.6± 1.4	4.7±0.2	7.5± 0.4	6.3± 0.1	+1.6
16:2ω4 (Palmitolenic)	4.5± 0.3	1.0±0.0	0.9± 0.0	1.1± 0.0	+0.1
18:0 (Stearic)	4.4± 0.4	5.8±0.0	2.4± 0.0	4.2± 0.5	-1.6
18:1ω9 (Oleic)	36.9± 0.8	28.5±0.2	30.8± 2.5	31.4± 0.1	+2.9
18:2ω9 (γ-Linoleic)	-	4.9±0.3	-	-	-
18:2ω6 (α-Linoleic)	11.0± 0.0	-	7.0± 0.3	7.2± 0.0	+7.2
18:3ω3 (α-Linolenic)	0.5 ±0.0	-	3.0±0.0	17.4± 0.0	+17.4
18:3 (γ-Linolenic)	-	18.4±0.1	-	-	-
20:0 (Arachidonic)	ND	0.3±0.0	ND	ND	-
20:1ω9 (Gadoleic)	ND	3.0±0.3	3.0± 0.0	3.9± 0.3	+0.9
20:3ω6	-	1.0±0.2	-	-	-
22:1ω11 (Erucic)	4.7± 0.4	-	ND	ND	-
20:4ω6 (Arachidonic)	ND	0.5±0.0	0.6± 0.0	0.8± 0.2	+0.3
20:5ω3 (EPA)	4.8± 0.5	2.1±0.1	10.0± 0.3	9.0± 0.8	+6.9
22:6ω3 (DHA)	ND	-	5.6± 0.4	2.3± 0.1	+2.3

Note: ND = Not Detected      EPA = (Eicosapentaenoic)      DHA = (Docosahexaenoic)

**Fatty acid analysis:** Fatty acid composition of RAC II (Reference *Artemia* cysts), AEP (*Artemia* Enrichment Product) and Great Salt Lake *Artemia* with and without AEP, was determined using gas chromatography. Crude lipids were estimated by Bligh and Dyer (1959) lipid extraction method as modified by Kates (1986). Fatty acid profile was determined using the protocol of the National Marine Fisheries Service (NOAA) (1988) for the analysis of marine fish oil. Fatty acids were saponified and esterified with boron trifluoride methanol (BF<sub>3</sub> methanol) reagent to form fatty acid methyl esters (FAME; AOAC, 1995). (Folch *et al.*, 1957), The FAMES were analyzed using Carlo Erba Strumentazione Series 4160 gas chromatograph. A 30 m fused silica glass capillary column (I.D. 0.32 mm) coated with SP-2330 0.2 mm thick film from Supelco Inc., Bellefonte, P.A., was used. The free fatty acids were detected by flame ionization detector. Helium gas with a flow rate of 25 ml/min at a column pressure of 0.57 kg/cm<sup>2</sup> was used as a carrier gas. Injector and detector temperature was 220°C. Oven temperature was held at 150°C for 8 min and programmed up to 190°C at 3°C with a linear velocity of 20 cm/min. Detector response was integrated and quantitated with a Hewlett-Packard 3390A integrator. After dilution with 10% chloroform, the samples

containing FAME, were injected and separated on a above described fused silica capillary column. Fatty acyl methyl esters were identified by comparison of their retention times to those of standards (Spelco Inc., Bewlfonte, P.A.). The results have been expressed as area percent Fatty Acid Methyl esters (FAME) (Table 1).

**Development of stress test and data collection:**

Twenty-five-day-old striped bass and 31 day old inland silverside larvae were exposed to 10, 20, 30 and 40‰ and 40, 60, 65, 70, 75, 80, 85 and 100‰ salinity respectively (Table 2, 3). Striped bass exposures were subdivided into a) 1-h post-feeding, b) 4-h post feeding and c) 8-h post feeding states. In 1-h post-feeding state, the fish were fed on *Artemia* nauplii to satiation. When sufficient nauplii were seen in the gut of the fish, they were harvested and exposed to the higher salinity levels mentioned above. Mortality was monitored every ½ h for 2 h, then monitored again after 15 h of exposure. In the 4-h post-feeding, larvae were fed as above but then were not disturbed for 4 h after feeding. Nauplii were then harvested and exposed to the same salinities as explained earlier. At this time nauplii in the gut were partially digested and still some nauplii were visible through the transparent gut. In the 8-h post feeding

Table 2: Growth and survival of striped bass and inland Silver sides at the end of 2nd and 3rd week of rearing period respectively under various feeding regimes

Species	Fed on	Initial weight (mg)	Final weight (mg)	Initial length (mm)	Final length (mm)	Survival (%)
Striped bass	Great Salt Lake <i>Artemia</i> naupli	2.8±0.3	16.63±0.8	2.7±0.31	13.58±0.3	85.0±4.5
Inland Silver sides	Un-enriched <i>Artemia</i> nauplii	5.2±0.3 <sup>a</sup>	32.0±2.1 <sup>b</sup>	11.0±1.0 <sup>c</sup>	18.4±1.5 <sup>d</sup>	97.2±1.5 <sup>e</sup>
	Enriched <i>Artemia</i> nauplii	5.3±0.4 <sup>a</sup>	33.1±1.9 <sup>b</sup>	11.0±1.5 <sup>c</sup>	19.5±2.0 <sup>d</sup>	98.1±1.2 <sup>e</sup>

Table 3: Mortality (%) of striped bass larvae exposed to higher salinities

Time (h)	Salinity ppt			
	10	20	30	40
<b>a) 1-h post-feeding</b>				
½	0	0	0	30±7.0
1	0	0	0	85±12.0
1 and ½	0	0	15±3.5	90±9.5
2	0	0	15±4.0	100
15	20±4.0	40±6.5	80±8.5	100
<b>b) 4-h post feeding</b>				
½	0	0	0	0
1	0	0	0	0
1 and ½	0	0	15±4.0	80±5.0
2	0	10±5.0	20±8.0	100
15	0	10±6.5	90±9.8	100
<b>c) 8-h post feeding</b>				
½	0	0	0	0
1	0	0	0	0
1 and ½	0	0	10±3.0	30±5.0
2	0	0	20±4.0	80±6.0
15	0	20±7.0	50±9.0	100

Total number of fish used = 10 fish/replicate x 3 replicates x 4 treatments x 4 stress tests  
= 360 fish

group, the larvae were fed as above but were collected after 8 h and exposed to the same salinity levels as in "a" and "b". There were 30 fish per treatment with three replicates in each treatment (salinity level). Silverside exposures were also further subdivided into a) range finder 1, b) range finder 2 and c) definitive test. In range finder 1, the silverside larvae from holding tank were exposed to salinities of 40, 60, 80 and 100 ppt. The survival was monitored during 2 h exposure. The data collected were used to set up the salinity range for range finder 2. In range finder 2, the overall salinity range was narrowed and fish was exposed to only 60, 70 and 80‰ salinity for 2 h. The survival data acquired from this test was used to arrange the salinity levels for the definitive test, in which the fish were exposed to 65, 70, 75 and 80‰ salinity. The various salinities used in each test are given in Table 3. There were three fish in each replicate three replicates for each treatment in range finder 1 and 5 fish in each replicate for range finder 2 and for definitive test. Replicate number was uniform throughout the stress test.

In both experiments sea water was evaporated by continuous heating to raise salt concentration up to desired level. The hot water was brought to room temperature and aerated vigorously. Where required, distilled water was added and required dilutions were prepared. The salinity levels were continuously monitored by a temperature compensated Refractometer. The fish were not netted but were collected individually from the aquaria with a small bowl. They were concentrated in a strainer immersed in rearing water. When a required number was achieved, they were transferred to a higher salinity treatment and held there for 2 h. The exposure time was further prolonged for striped bass (Table 2). The survival was monitored at ½ h intervals during the 2 h exposure. Dead fish were removed and counted (Table 2, 3).

**Statistical analysis:** Mortality values were analyzed by one-way ANOVA using statistical software (SPSS 11.0 version). Duncan's Multiple Range Test was used to compare means between treatments for their statistical significance. Differences were considered significant at  $p < 0.05$ .

## RESULTS

**Fatty acid composition of enriched *Artemia* nauplii:** The fatty acid composition of *Artemia* nauplii has been summarized in Table 1. The fatty acids such as 15:0, 17:0, 16:1 $\omega$ 7, 16:2 $\omega$ 4, 18:1 $\omega$ 9, 18:2 $\omega$ 6, 18:3 $\omega$ 3 and 20:1 $\omega$ 9 showed considerable increments but elevated levels of Polyunsaturated Fatty Acids (PUFA) arachidonic acid (20:4 $\omega$ 6), eicosapentaenoic acid (20:5 $\omega$ 3) and docosahexaenoic acid (22:6 $\omega$ 3) were more prominent.

Fatty acids 14:0, 16:0 and 18:0 exhibited decline in concentrations. Fatty acids 13:0, 18:2 $\omega$ 9, 18:3, 20:0 and 20:3 $\omega$ 6 were though present in *Artemia* nauplii before enrichment but were totally lost after enrichment. Pentadecanoic acid (15:0) could not incorporate itself at all.

**Striped bass:** Striped bass was reared on RAC nauplii. Survival was 85%. During the 2 weeks of the feeding trial fish increased in mass from  $2.8 \pm 0.3$  mg to  $16.63 \pm 0.8$  mg (means  $\pm$  SEM) (Table 2). No deformity or disease was observed during rearing. After two weeks of *Artemia* feeding, fish were exposed to various salinity levels to determine their salinity tolerance. Each salinity levels higher than 20‰ gave some mortalities within two hours but there was considerable variation in mortality data when it was compared from salinity to salinity. Significant ( $p < 0.05$ ) differences were apparent not only at different salinities but also over exposure time (Table 3). Increased exposure time significantly ( $p < 0.05$ ) increased mortality, but this increase was less perceptible at lower salinities. Four hour post-feeding and 8-h post feeding fish performed significantly ( $p < 0.05$ ) better than 1-h post-feeding fish (Table 3a, 3b and 3c).

**Inland Silver sides:** Neither any deformity or disease was observed, nor was there any difference in growth and survival between treatments at the end of the feeding trials. Enrichment of *Artemia* did not induce any additional effects upto this stage (Table 2). However, after three weeks when fish were exposed to various elevated levels of salinities to determine their resistance capability, pronounced differences were observed. All the fish survived at 60‰ salinity and below and almost all were dead at 80‰ and above. Mortality counts were similar in range finder 1 [Table 4(i)] regardless of the nutritional status of food organisms (enriched or un-enriched). Range finder 1 pointed out that 60 ‰ or lower salinity did not kill the fish in 2 h. But 80‰ salinity killed all individuals. Both these extremes were therefore not suitable to detect differences in physiological condition of fish larvae. Therefore, in range finder 2 extreme salinity levels (40‰ and 100‰), were dropped and then larvae were exposed to 60, 70 and 80‰ salinities. The treatment groups were significantly ( $p < 0.05$ ) different at 70‰ but not at 60 and 80‰ in range finder 2 [Table 4(ii)]. In the definitive test, significant differences between treatments were observed at 65‰ [Table 4(iii)], with fish fed AEP-enriched nauplii showed significantly better survival. No differences were observed at 70, 75, or 80‰ after 2 h. Number of survivors significantly ( $p < 0.05$ ) declined and all fish died within the first half of experiment.

Table 4: Mortality (%) of inland Silver sides exposed to higher salinities fed AEP-enriched vs. unenriched *Artemia* nauplii

	----- Salinity (ppt) -----			
<b>i) Range finder 1</b>				
Time (h)	40	60	80	100
<b>a) Unenriched</b>				
½	0	0	100	100
1	0	0	100	100
1 and ½	0	0	100	100
2	0	0	100	100
<b>b) AEP-enriched</b>				
½	0	0	100	100
1	0	0	100	100
1 and ½	0	0	100	100
2	0	0	100	100
<b>ii) Range finder 2</b>				
Time (h)	60	70	80	
<b>a) Unenriched</b>				
½	0	0	0	
1	0	10±10	10±10	
1 and ½	0	20±0	30±10	
2	0	20±0	30±10	
<b>b) AEP-enriched</b>				
½	0	0	0	
1	0	0	20±10	
1 and ½	0	0	20±10	
2	0	0	20±10	
<b>iii) Definitive test</b>				
Time (h)	65	70	75	80
<b>a) Unenriched</b>				
½	0	0	0	0
1	0	10±10	20±20	30±10
1 and ½	40±40	80±20	60±20	70±10
2	80±20	89±10	90±10	100
<b>b) AEP-enriched</b>				
½	0	0	0	0
1	0	0	10±6	100
1 and ½	10±10	60±10	60±10	100
2	30±10	90±10	90±10	100

Total number of fish used

i) Range Finder 1 = 3 fish/replicate x 3 replicates x 4 treatments x 2 groups = 72 fish

ii) Range Finder 2 = 5 fish/replicate x 3 replicates x 3 treatments x 2 groups = 90 fish

iii) Definitive test = 5 fish/replicate x 3 replicates x 4 treatments x 2 groups = 120 fish

Total i + ii + iii = 282 fish

## DISCUSSION

The present studies defined a salinity range for 25 day old striped bass in which differences between maximum survival and mortality can be ascertained. These studies further confirmed that immediate exposure or transfer to a stressful environment, after feeding, can lead to high mortality. Pecha *et al.* (1983) demonstrated in his studies that fish with full digestive tracts needed more oxygen to combat stress due to production of undigested excreta which took much of the oxygen of the water. Fish hence became more susceptible to stress. Ammonia built up due to protein metabolism of oxygen uptake inhibited the ability of hemoglobin to combine with oxygen, altering the oxygen carrying-capacity of the blood (Comogolio *et al.*, 2008). The metabolism of blue gourami was found to decrease with increasing duration of starvation (Chow *et al.*, 1994). The stress resistance of guppies starved for 1 day was significantly higher than that of fish starved for two days indicating that starvation

for this fish should not exceed 1 day (Chuan Lim *et al.*, 2003). In our studies those striped bass whose feed was withheld long enough (15 h) and they have voided their stomachs and intestine, did not face this difficulty and survived longer than their counterparts. The fish were reared on Reference *Artemia*, it is not clear that higher or lower levels of HUFA in the diet could affect tolerance ability of striped bass to a stressful environment.

Sorgeloos *et al.* (1988) reported the essentiality of HUFA for culture of larval fish. Lisac *et al.* (1986), Franicevic *et al.* (1987) and Watanabe *et al.* (1982) confirmed HUFA requirement for European sea bass, sea bream and other bream species for growth, survival, advancement in metamorphosis (Babitha *et al.*, 2006) and during activity test. Forty day old Japanese flounder, *Paralichthys olivaceus*, has shown better growth and survival and increased tolerance when exposed to higher salinity levels when fed on HUFA enriched

*Artemia* nauplii (Furuita *et al.*, 1999). DHA, however, showed superiority over EPA in inducing resistance to stress. Like other fish species, striped bass has shown better performance in growth and survival when fed on *Artemia* nauplii containing HUFAs (Webster and Lovell, 1990). Like Asian sea bass (Dhert *et al.*, 1990) striped bass survival under stressful conditions might be improved by feeding diets high in HUFA contents. But presently it will not be discussed further for striped bass because their effect was not tested. In the current studies response of fish was observed when exposed to higher salinities and its relationship was developed between duration from last feeding with elevated salinity level.

Unlike striped bass, *Menidia beryllina*, were fed on HUFA-deficient and HUFA-rich *Artemia* nauplii. Then salinity range for salinity stress tests for this species was determined. Moreover nutritional effectiveness of HUFA supplementation in *Menidia* food was evaluated. Prior to the stress test, there were no differences in growth and survival between *Menidia* larvae fed HUFA-deficient or HUFA-rich *Artemia*. However, fish fed high HUFA diet exhibited better survival in the salinity stress test than did those fed un-enriched *Artemia*. To detect quality of *Penaeus monodon* post larvae fed on n-3 HUFA enriched *Artemia*. Rees *et al.* (1994) subjected the larvae to 0, 5 and 10‰ salinity and found that the n-3 HUFA enriched *Artemia* fed groups resisted the stress significantly ( $p < 0.05$ ) better than those of fed un-enriched *Artemia*. Citarasu *et al.* (2002) after 30 days of culture observed marked differences in the stress resistance ability of *Penaeus* post larvae when exposed to osmotic shock, pH and formalin stress. The un-enriched *Artemia* fed group succumbed to death within 80 min while the enriched *Artemia* fed group was able to tolerate it to a maximum of 140 min. The beneficial effect of HUFA supplementation in the diet on survival to salinity stress test is partially related to modification of fatty acid composition of gills and to a larger gill area which in turn enhances osmoregulatory mechanisms, namely  $\text{Na}^+/\text{K}^+$  ATPase and carbonic anhydrase activities (Palcios *et al.*, 2004). Examination of fatty acid levels in live feeds and larval tissue confirmed the physiological incorporation of fatty acids relative to dietary levels. Better resistance was noticed in *Penaeus indicus* fed on HUFA enriched *Artemia* (Immanuel *et al.*, 2001). Studying the effect of n-3 HUFA, Horstmark *et al.* (1987) demonstrated that erythrocytes of rats fed HUFA rich cod liver oil achieved a higher resistance to hypo osmotic shock, an effect that probably resulted from a higher incorporation of n-3 HUFA in cell membranes. If similar phenomenon also occurs in crustaceans, the better resistance of fish fed HUFA enriched *Artemia* may be attributed to the increased osmotic resistance of their cells, delaying the onset of irreversible damage in some essential tissues (Rees *et al.*, 1994). When sea bream and Atlantic salmon (*Salmo salar*) were subjected to

confinement to induce a stress response (McCormick, 2001; Iversen *et al.*, 2005)), the fatty acid composition of the gills was affected. The PUFA level decreased in gill tissue while saturates and mono un-saturates decreased. Catechol amines released due to this physiological turnover (Koven *et al.*, 2003) can lead to an increased permeability of the branchial epithelium leading to influx of ions in fish in a hyper-osmotic environment. This in turn stimulates the ionic extrusion by the chloride cells, located mainly in the opercula and gills. Larval summer flounder, *Paralichthys dentatus* fed on fatty acid enriched rotifers were better able to survive the salinity tolerance test (Willey *et al.*, 2004). Jalali *et al.* (2008) observed better growth, survival and stress resistance in those beluga (*Huso huso*) larvae which were fed on HUFA and vitamin E enriched *Artemia urmiana*. Probably during other stressful conditions such as transportation, transfer from hatchery to culture ponds or when fish are under other stressful conditions (e.g. oxygen deficiency, poor feeding and drastic temperature fluctuations) high HUFA-enriched diets could improve the physiological fitness of the larvae and make them more resistant to unfavourable environments (Dhert *et al.*, 1990).

The present studies have demonstrated marked changes in the stress response due to feeding HUFA enriched *Artemia* nauplii for 3 weeks which emphasizes the physiological impact of these fatty acids. It can therefore be concluded that where extensive fish handling or other stressful conditions are expected, incorporation of HUFA in the diet is highly important. Moreover, a practical tool (stress test) has been developed during these studies which can provide very useful criteria for research purposes to evaluate diet effectiveness and could be beneficial for aquaculturists to evaluate the physical condition of the larvae.

## REFERENCES

- AOAC, 1995. Official Methods of Analysis of AOAC International, vol. 1, 16th Edn. (Ed. P.a. Cunniff). AOAC Int., Arlington, VA.
- Arellano, E., 1990. Fatty acid composition of wild and cultured *Penaeus vannamei* as a method to evaluate post larval quality. World Aquaculture Society 21<sup>st</sup> Annual Conference, pp: 150.
- Babitha Rani, A.M., A.K. Reddy and N.P. Sahu, 2006. Growth enhancement and survival of *Macrobrachium rosenbergii* fed *Artemia* nauplii enriched with cod liver oil and/or *Lactobacillus*. The Israeli J. Aquaculture-Bamidgeh, 58: 183-190.
- Bauman, R.H. and D.R. Jamandre, 1990. A practical method for determining quality of *Penaeus monodon* (Fabricious) fry for stocking in grow-out ponds. In: Technical and Economic Aspects of Shrimp Farming (Eds. M.B. New, H. Saram and T. Sing), Proceedings of the Aquatec. 90' Conference, Infish Publications. Kuala Lumpur, pp: 124-137.

- Bengtson, D.A., A.D. Beck and K.L. Simpson, 1985. Standardization of the nutrition of fish in aquatic toxicological testing. In: Nutrition and Feeding in Fish (Eds. C.B. Cowey, A.M. Mackie, J.B./Bell). Academic Press, London, pp: 431-446.
- Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37: 911-917.
- Chuan Lim, L., P. Dhert and P. Sorgeloos, 2003. Recent developments and improvements in ornamental fish packing systems for air transport. *Aquac. Res.*, 34: 923-935.
- Citarasu, T., M. Micheal Babu, R. Raja Jeya Sekar and M. Peter Marian, 2002. Developing Artemia enriched herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. *Asian Fish. Sci.*, 15: 21-32.
- Chow, P.S., T.W. Chen and L.H. Teo, 1994. Oxygen consumption rates of *Trichogaster trichopterus* (Pallas) in relation to starvation, size and sex. In: The Third Asian Fisheries Forum (Eds., L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheon, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang., K.F. Shim and C.H. Tan) Asian Fisheries Society, Manila, Philippines, pp: 884-887.
- Comogolio, L., J. Goldsmit and O. Amin, 2008. Starvation effects on physiological parameters and biochemical composition of the hepatopancrease of the southern king crab, *Lithodes santolla* (Molina, 1782). *Revista de Biologia Marina y Oceanografia*, 43: 345-353.
- Dhert, P., P. Lavens, M. Duray and P. Sorgeloos, 1990. Improved larval survival at metamorphosis of Asian sea bass, *Lates calcarifer* using W3-HUFA enriched live food. *Aquaculture*, 90: 63-74.
- Folch, J., M. Lees and G.H. Stoane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.*, 226: 497-506.
- Franicevic, V., J. Lisac, J. Buble, P. Leger and P. Sorgeloos, 1987. Etude internationale sur 1, *Artemia*. XLII. L'effect de la qualite nutritionelle de 1' Artesia sur la croissance et la survie des larves du loup (*Dicentrarchus labrax*) dans une ecloserie commerciale. In: B. Loix (Editor). Production in Marine Hatcheries. FAO-MEDRAP, Rovinj-Zadar, Yugoslavia, pp: 139-145.
- Furuita, H., K. Konishi and T. Takeuchi, 1999. Effect of different levels of ecosapentaenoic acid and docosahexaenoic acid in *Artemia* nauplii on growth, survival and salinity tolerance of larvae of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 170: 59-69.
- Horstmark, A.T., E. Lystad and A. Haug, 1987. Cod liver oil increases osmotic resistance of blood cells. *Nutr. Rep. Int.*, 36: 613-616.
- Immanuel, G., A. Palavesam and M. Petermarian, 2001. Effects of feeding lipid enriched *Artemia* nauplii in survival, growth, fatty acids and stress resistance of post larvae *Penaeus indicus*. *Asian Fish. Sci.*, 14: 377-388.
- Iversen, M., B. Finstad, R.S. Mckinley, R.A. Eliassen, K.T. Carlsen and T. Evjen, 2005. Stress response in Atlantic salmon (*Salmo salar* L.) smolts during commercial well boat transports and effects on survival after transfer to sea. *Aquaculture*, 243: 373-382.
- Jalali, M.A., S.A. Hussein and M.R. Imanpour, 2008. Effect of vitamin E and highly unsaturated fatty acid-enriched *Artemia urmiana* on growth performance, survival and stress resistance in Beluga (*Huso huso*) larvae. *Aquac. Res.*, 39: 1286-1291.
- Kates, M., 1986. Lipid Extraction Procedures. In: Techniques of Lipidology; Isolation, Analysis and Identification of Lipids. Amsterdam: Elsevier Press, pp: 347.
- Kontara, E.K., G. Merchie, A. Neilis, A. Dee Leenheer and P. Sorgeloos, 1995. Improved larviculture output of the post larval shrimp *Penaeus vannamei* through supplementation of 2-ascorbyl-2-polysorbate in the diet. In: Larvi 95 (Eds. P. Lavens, E. Jaspers, I. Roelants) Spec. Publ. 24, Eur. Aquac. Soc., Ghent, pp: 230-234.
- Koven, W.M., R.D. Van Anholt, S. Lutsky, I. Ben Atia, O. NIXON, B. Ron and A. Tandler, 2003. The effect of dietary arachidonic acid on growth, survival and cortisol levels in different-age gilthead sea bream larvae (*Sparus auratus*) exposed to handling or daily salinity change. *Aquaculture*, 228: 307-320.
- Kuhlmann, D., G. Quantz and U. Witt, 1981. Rearing of turbot larvae (*Scophthalmus maximus*) on cultured food organisms and post-metamorphosis growth on natural and artificial food. *Aquaculture*, 23: 183-196.
- Leger, P., D.A. Bengtson, K.L. Simpson and P. Sorgeloos, 1986. The use and nutritional value of *Artemia* as a food source. *Oceanography Marine Biology Annu. Rev.*, 24: 521-623.
- Lisac, D., V. Franicevic, Z. Vejmelka, J. Buble, P.H. Leger and P. Sorgeloos, 1986. International study on *Artemia*. XLIII. The effect of live food fatty acid content on growth and survival of sea bream (*Sparus aurata*) larvae. Paper presented at the Conference Ichthyopathology in Aquaculture, 21-24 October, Inter-University Center, Dabrovnik.
- McCormick, S.D., 2001. Endocrine control of osmoregulation in teleost fish. *Am. Zool.*, 41: 335-347.
- NOAA, 1988. Fatty acid composition. In: Biomedical Test Materials Program: Analytical Methods for the Quality Assurance of Fish Oil. 9 Eds. F.m. Vandolah and S.B. Galloway), Charleston, SC., U.D. Department of Commerce, pp: 6-11.



- Pecha, O., R. Berka and J. Kouril, 1983. Preprava pludku v polyetylenoych vacich (Fry transport in polyethylene bags) Ser. Metod. VURH Vodnany, (10): pp: 16.
- Placios, E., A. Bonilla, A. Perez, I.S. Racotta and R. Civera, 2004. Influence of highly unsaturated fatty acids on the responses of white shrimp (*Litopenaeus vannamei*) post larvae to low salinity. J. Exp. Marine Biol. Ecol., 299: 201-215.
- Rees, J.F., K. Cure, S. Piyatirativorakul, P. Sorgeloos and P. Menuveta, 1994. Highly unsaturated fatty acid requirements of *Penaeus monodon* post larvae and Experimental approach based on *Artemia* enrichment. Aquaculture, 122: 193-201.
- Santhanakrishnan, G. and M. Visvakumar, 1995. Present status of marine shrimp seed production of future prospectus to meet the demand with suitable Technological Development, 43-48. In: INDAQUA '95, MPEDA publication, Cochin India, pp: 134.
- Sorgeloos, P., P. Leger and P. Lavens, 1988. Improved larval rearing of European sea bass and Asian sea bass, sea bream, mahi mahi, siganid and milk fish using enrichment diets for Brachionus and Artemia. World Aquac. Soc., 19: 77-88.
- Tackaert, W., P. Abelin, P. Dhert and P. Sorgeloos, 1989. Stress resistance in post larval Penaeid shrimp reared under different feeding procedures. J. World Aquac. Soc., 20: 744.
- Watanabe, T., F. Oowa, C. Kitajima and S. Fujita, 1978. Nutritional quality of brine shrimp, *Artemia salina*, as a living food from the view point of essential fatty acids for fish. Bull. Japanese Soc. Scientific Fish., 44: 1115-1121.
- Watanabe, T., F. Oowa, C. Kitajima and S. Fujita, 1980. Relationship between dietary value of brine shrimp *Artemia salina* and their content of W3 highly unsaturated fatty acids for fish. Bull. Japanese Soc. Scientific Fish., 46: 35-41.
- Watanabe, T., M. Ohta, C. Kitajima and S. Fujita, 1982. Improvement of dietary value of brine shrimp (*Artemia salina*) for fish larvae by feeding them on W3 highly unsaturated fatty acids. Bull. Japanese Soc. Scientific Fish., 48: 1775-1782.
- Webster, C.D. and R.T. Lovell, 1990. Quality evaluation of four sources of brine shrimp *Artemia* sp. J. World Aquac. Soc., 22: 180-185.
- Willey, S., D.A. Bengtson and M. Harel, 2004. Arachidonic acid requirements in larval summer flounder, *Paralichthys dentatus*. Aquaculture Int., 11: 131-149.