www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 423-429 (2010) DOI: 10.4194/trjfas.2010.0317

The Use of Eugenol as an Anesthetic in Transportation of With Indian Shrimp (*Fenneropenaeus indicus*) Post Larvae

Sohrab Akbari[1,*], Mohammad J. Khoshnod[1], Hamid Rajaian[2], Mohammad Afsharnasab[3]

[1] Shiraz University, Veterinarian Faculty, Department of Clinical Studies, Po Box: 71345-1731, Shiraz, Iran.
[2] Shiraz University, Department of Basic Sciences, Veterinarian Faculty, Po Box: 71345-1731, Shiraz, Iran.
[3] Iranian Fisheries Research Organization, P.O. 14155-3464, Tehran, Iran.

* Corresponding Author: Tel.: +98.711 6138736; Fax: +98.711 2286940;
E-mail: esattopkara@gmail.com

Received 09 January 2009
Accepted 04 March 2010

Abstract

The safe margin of eugenol for sedation of the healthy post larvae (PL) of white Indian shrimp (*Fenneropenaeus indicus*) was determined by estimating the median lethal concentration of eugenol during 24 hours (24hLC50). Sedation concentration of eugenol was obtained experimentally by considering behavioral factors of PLs such as response to indirect stimuli and changes in water quality parameters. Applying the estimated concentrations of eugenol for 12 hours transportation of PLs, biometric parameters of sedated animals were compared with the control after 96 hours post transportation at $P<0.05$. The 24hLC50 of eugenol for experimental PLs was found to be 5.2 mg/L with minimum confidence limit of 5.0 mg/L and maximum confidence limit of 5.3 mg/L. The sedation concentrations for PLs varied from a minimum level of 1.3 mg/L to a maximum level of 3.7 mg/L. During 24 hours sedation, significant reductions were found in the oxygen consumption of PLs where as no significant changes occurred in their ammonia secretion. There was no change in biometric parameters in sedated animals after 12 hours transportation. The results indicate that eugenol can be used as a sedative during transportation of PLs of *F. indicus* and concentration of 1.3 mg/L considered is safe which showed the lowest induction of bottom sitting.

Keywords: Transportation, Eugenol, Anesthesia, white Indian shrimp, *Fenneropenaeus indicus*.

Bir Anestezik Olarak Ögenolun, Hint Karidesi (*Fenneropenaeus indicus*) Post Larvalarının Taşınmasında Kullanılması

Özet

Beyaz Hint karidesinin (*Fenneropenaeus indicus*) sağlıklı post-larvalarının (PL) sakinleştirilmesi (sedasyon) için güvenli ögenol sınırı, 24 saat boyunca (24h LC50) orta seviye ölümcül ögenol konsantrasyonu belirlenmiştir. Dolaylı uyaranlar ve su kalite parametrelerine cevap gibi larva davranış faktörleri dikkate alınarak ögenolun sakinleştirici konsantrasyonu deneysel olarak elde edilmiştir. Post-larvaların 12 saat için taşınması amacıyla tahmini ögenol konsantrasyonu uygulayarak, sedasyon altındaki hayvanların biyometrik parametreleri $P<0,05$'te, taşındıktan 96 saat sonra kontrol ile karşılaştırılmıştır. Denek post-larvalarda 24hLC50 ögenol, 5,0 mg/L'lik minimum ve 5,3 mg/l'lik maksimum güven sınırıyla uygulama konsantrasyonu 5,2 mg/L olarak bulunmuştur. PL'ler için sedasyon konsantrasyonları, minimum 1,3 mh/L ile maksimum 3,7 mg/L arasında değişmiştir. 24 saatlik sedasyon boyunca PL'lerin oksijen tüketiminde anlamlı düşüşler tespit edilmiştir. Taşındıktan 12 saat sonra sedasyon altındaki hayvanlarda biyometrik parametrelerde hiçbir değişiklik olmamıştır. Sonuçlar, *F. İndicus* post-larvaların taşınmasında sakinleştirici olarak ögenolun kullanılabileceğini ve 1.3 mg/L konsantrasyonun güvenli olduğunu göstermiştir.

Anahtar Kelimeler: Transportation, Eugenol, Anesthesia, white Indian shrimp, *Fenneropenaeus indicus*.

Introduction

Stress induced by changes in environmental parameters require homeostatic regulation that brings about behavioral and physiological alterations in aquatic animals (Akbari and Shariff, 2003; Li and Brouwer, 2007). In aquaculture, various strategies have been applied to relief the aquatic animals from regular stressful conditions during transportation. Anesthesia has been introduced as an applicable way to prevent stress in aquatic animals for long times. For induction of anesthesia, tricaine methane sulfonate (MS-222) has received more attention than any other chemicals because it is the most commonly used fish anesthetic in North America. The performance of MS-222 in fish anesthesia has still received high attentions

in research documents (Cordova and Braun, 2007; Rombough, 2007). A concentration of anesthetic that produces a light sedation (i.e. stage I of anesthesia according to Jolly *et al*., 1972) is desirable for transporting fish. However, Summerfelt and Smith (1990) stated that the ideal level of sedation for fish transport is referred to as deep sedation and includes loss of reactivity to external stimuli, decrease in metabolic rate, but maintenance of equilibrium that consistent with stage II anesthesia in fish.

Most operations in crustacean's culture can be conducted without anesthesia, although the rapid movement of shrimp may cause handling problems and their cannibalistic nature and sharp rostrum can be a problem during transportation. Consequently, there has been some interest in investigating shrimp anesthetics, particularly for transport. Shrimp respond differently to anesthesia than finfish. For example, MS-222 is not effective on crustaceans such as freshwater prawn, *Macrobrachium rosenbergii* (Coyle *et al*. 2005). A higher concentration of MS-222 is required to induce anesthesia in *Peaaeus indicus*, than that in fish (Akbari and Khajavi, 2004). Aqui-S has been reported to be effective in freshwater prawn, but only at concentrations of 5 to 10 times greater than those used in finfish (Coyle *et al*., 2005). Carbon dioxide is an effective anesthetic for most crustaceans. It is most frequently dispensed as a mixture of baking soda and acetic acid. Cooling is also an effective way to immobilize shrimp, but one must be careful because cooling can kill the animals.

Eugenol has been widely used as a pain killer in human dentistry and as a food flavoring. Eugenol (C_{10} H_{12} O_2) is the major constituent (70 to 90 percent by weight) of clove oil, but it contains a wide range of other compounds that impart its characteristics like odor and flavor. It is an effective anesthetic in fish (Harper, 2003) and did not induce primary and secondary stress responses in fathead minnows, *Pimephales promelas* compared to MS-222 (Palic *et al*., 2006). Clove oil (eugenol) is also an effective anesthetic for shrimp (Akbari and khajavi, 2004; Coyle *et al*., 2005). The major advantage of eugenol is that it is inexpensive and not unpleasant to work with. Due to advantages of eugenol and its acceptable efficiency in shrimp anesthesia compared to other anesthetics and lack of basic data, this study was conducted to investigate the sedative properties of eugenol in post larvae (PL) of *F. indicus* which is the most target stage of animal for transportation. The median lethal concentration (LC50) of eugenol for the PLs was also investigated to find out the safety margin of eugenol for its practical application in transportation of the experimental animal.

Materials and Methods

Experimental Design

The experiments were conducted in a shrimp hatchery located in Delvar, Bosher Province, IR Iran. Commercial post larvae at the stages of PL13 or PL14 prepared for transportation to grow-out farms were used in these experiments. Quality of the PLs was tested, using stress test and physical investigations based on Akbari and Tokhm Afshan (2001).

Eugenol (Dentaires S. A. Vevey, Suisse) was used as the anesthetic. It was used to estimate its 24hLC50 and sedative concentration for the experimental animal. Plastic bags were used as the container of PLs for all experiments. Each container was filled with one liter of filtered and acclimated seawater. Comparison method were used for counting of 500 PLs which were packed in each experimental and control containers. After packing the PLs in each container seventy percent of its volume was overflowed with oxygen. The containers were tied with rubber bands separately and set for observation of mortalities during 24hLC50 assay and behavioral factors such as inactivation, and response to indirect stimuli during sedation experiments. Each experiment was preformed in triplicates with its own separated control.

Estimation of 24hLC50 of Eugenol for PLs of *F. indicus*

This experiment was performed based on Akbari *et al*. (2004). In the first step, the highest exposure concentration of eugenol causing 50% mortality in PLs during 4 hours was estimated by a primary test. Based on primary test, the highest exposure concentration of eugenol for estimation of 24hLC50 in the PLs of *F. indicus* was 32 mg/l. In this experiment, 3000 PLs were divided equally in six plastic bags which set in five experimental containers and one control. The PLs in the experimental containers were exposed to 2, 4, 8, 16, and 32 mg/l of eugenol. Mortalities in each experimental container were collected at six equal intervals during 24 hours. Based on the mortalities in each experimental container, 24hLC50 of eugenol for experimental animal was estimated by the use of trimmed Spearman-Karber method (Spraque, 1990; Akbari *et al*., 2004). There was no any mortalities in the control containers.

Determination of Sedative Concentration of Eugenol for PLs of *F. indicus*

This experiment was preformed on three groups of 2,000 PLs. Animals were equally divided in three experimental and one control containers. Based on a primary experiment, 75% of the minimum confidence limit of 24hLC50 of eugenol which estimated as 5.0 mg/L was used as the highest exposure concentration of eugenol to estimate its sedative concentration. In this experiment PLs were exposed to three concentrations of eugenol as 3.7, 2.5, and 1.3 mg/L. The experiment was preformed during 24 hours

motionless test using the Summerfelt and Smith (1990) criteria for the test of anesthesia in aquatic animal. From behavioral point of view in the experimental PLs such as inactivation and response to indirect stimuli during sedation, data were obtained from each container. Changes in water quality parameters, such as dissolved oxygen and unionized ammonia in closed containers resulting from metabolic activities of animals were compared between the seawater in control and the experimental containers at the end of 12 hours active transportation in sedation condition. Dissolved oxygen was measured by the Winkler method (Parsons *et al.*, 1984; Gowen *et al*., 2007). Unionized ammonia was measured using phenate method adapted for seawater analysis (APHA 1992). During transportation, PLs in experimental containers were exposed to three concentrations of eugenol (3.7, 2.5 and 1.3 mg/L). The PLs were transported for 12 hours in a regular condition used for transport of the PLs to grow-out ponds. They were transported back to the hatchery for further investigations within the initial proposed time of 12 hours. In the hatchery PLs were transferred separately to each ten liter plastic pails, filled with fresh seawater and aerated. The changes in biometric parameters (standard length, gut to muscle ratio in the sixth abdominal segment and the number of dorsal spines of rostrums) were compared between 100 samples of each experimental and their control PLs during 96 hours in four equal intervals, using Akbari and Togk afshan (2001) critera for standardization of healthy PLs of shrimp in hatcheries. The PLs in each ten liter pails were fed according to the regular feeding program of the hatchery and 100% of the exposure seawater of the pails was daily replaced during the experiment. All data were compared statistically using t-test confidence at $P<0.05$ as significant level.

Results

Quality of the experimental PLs was confirmed by salinity stress test. In this test, there was no mortality in PLs as they exposed to 5 ppt salinity during one hour. The gut to muscle ratio in the sixth abdominal segments of the experimental PLs which reflect their quality were 25-33% and the number of dorsal spines of their rostrums were 3-5 as the Min.-Max. values, respectively.

The 24hLC50 of Eugenol for PLs of *F. indicus*

Mortality of the experimental PLs exposed to various concentrations of eugenol during LC50 test are shown in Table 1. Mortalities counted at each 4 hour intervals during 24 hours for five different concentrations and one control vary based on duration of exposure concentration of the eugenol.

The LC50 of eugenol for experimental PLs for each four hour interval are shown in Table 2. The mean 24hLC50 of eugenol found to be 5.8 mg/L with minimum (Min.) confidence limit of 5.0 mg/L and maximum (Max.) confidence limit of 5.3 mg/L (Table 2).

A negative correlation exists between LC50 values of eugenol for the PLs of *F. indicus* and the exposure time of animal to the toxicant. The values of LC50s are significantly obvious till sixteen hours after the initial time of toxicity test. After sixteen hours no significant differences could be seen till the end of experiment (i.e. at 24 hour). The low and the high margins of LC50s for each time are shown as the min. and the max. confidence limits of each LC50.

Table 1. Number of mortalities of the PLs of *F. indicus* (n = 500) exposed to different concentrations of eugenol in a 24hLC50 test. Data are presented in triplicates

Concentration of eugenol (mg/L)	No. ofreplicatetest	The number of shrimp mortalities at each times (h).						
		0	4	8	12	16	20	24
32	1	0	500	-	-	-	-	-
	2	0	500	-	-	-	-	-
	3	0	500	-	-	-	-	-
16	1	0	400	100	-	-	-	-
	2	0	450	50	-	-	-	-
	3	0	450	40	10	-	-	-
8	1	0	300	170	30	-	-	-
	2	0	300	50	50	-	-	-
	3	0	250	50	50	100	-	-
4	1	0	50	50	50	50	-	-
	2	0	50	-	-	-	-	-
	3	0	25	25	-	25	-	25
2	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Control	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0

Table 2. The LC50 values of eugenol and it's Min. - Max. confidence limits for the PLs of *F. indicus*. Data are the mean of triplicate tests

Time (h)	LC50 (mg/L)	Min. confidence limit (mg/L)	Max. confidence limit (mg/L)
4	7.9	7.5	8.2
8	6.2	6.0	6.4
12	5.7	5.4	5.9
16	5.2	5.1	5.4
20	5.2	5.1	5.4
24	5.0	5.2	5.3

Sedative Concentration of Eugenol for PLs of *F. indicus*

The first consequence in induction of sedation was bottom sitting of the animals while exposed to eugenol. Bottom sitting in the experimental PLs was observed but not permanently. They sit down on the bottom of the containers in various duration based on the concentration which were exposed (Table 3). The PLs showed different duration of resting, but all experimental PLs got up from sitting after 12 hours exposure to various sedative concentrations and swam regularly. However, there were no mortalities in the PLs population exposed to various sedative concentrations and the control. Minimum sitting time was observed in the PLs exposed to 1.3 mg/L of eugenol. The PLs exposed to this concentration were got up from sitting after 4 hours exposure to the sedative.

Responses to indirect stimuli as a basic marker of sedated PLs were based on the concentrations of exposure sedative. Based on the deepness of sedation the PLs took action by various numbers of knocks which played on the external cover of the containers. Table 4 shows the degree of sedation of PLs indicated by the marks defined under the presented table.

Changes in the water quality of the containers were detected after 12 hours exposure to sedation concentrations of eugenol during transportation. There were no significant changes for ammonia-N in any of the experimental containers compared to the control, but based on the concentration of the exposure eugenol dissolved oxygen in experimental containers varied significantly compared to the control (Table 5).

Discussion

The need for some means of immobilizing in aquatic animals without harm to the subject has long been recognized. Documents show that basic and applied researches have focused on immobilization of fish as the most applicable aquatics subjected to aquaculture. Anesthesia as a method of immobilizing is now a common practice in fish. The use of anesthetics could possibly improve transport survival; however, to date anesthetic agents have not been briefly evaluated for use in shrimp such as what have been defined for fish.

Two major constraints in the commercial culture of shrimp are poor survival during live transportation of PLs to grow out ponds, and live transportation of harvested bloodstocks to distant hatcheries due to the territorial and cannibalistic nature of shrimp. In the case of shrimp anesthesia, two experiments have been carried out with juvenile of freshwater shrimp, *Macrobrachiurn rosenbergii* by Coyle *et al*. (2005) to compare the effectiveness of anesthetics usually used in fish.

The first experiment was designed to find out the most promising applicants between MS-222, 2-phenoxyethanol, quinaldine sulfate (quinaldine), clove oil, and Aqui-S. MS-222 and 2-phenoxyethanol were determined to be ineffective on shrimp at all rates tested. Clove oil and Aqui-S induced anesthesia faster and at lower concentrations than quinaldine. At the highest treatment rate (300 mg/L) mortality rates in the Aqui-S, quinaldine and clove oil treatments were found to be 60%, 13% and zero percent respectively following one hour exposure to this concentration. Based on these data, Aqui-S and clove oil at a 100 mg/L may be suitable anesthetic for freshwater shrimp. In another study which compared the anesthetic effects of MS-222 and eugenol on *F. indicus* juvenile, eugenol showed more profound anesthetic effects than MS-222. The later induced anesthesia in *F. indicus* at a high concentration of 3,700 mg/L while at a low concentration of 22.5 mg/L, eugenol was adequate to induce anesthesia in the same shrimp (Akbari and Khajavi, 2004). The preceding documents point toward clove oil or its active extract (eugenol) as the right medicine for induction of anesthesia in shrimp. Regarding to the deep anesthetic effect of eugenol, research documents and the practical aspects show parallel results in fish and shrimp. But due to induction of light anesthetic effect of eugenol which induces sedation, there are a few documents in fish and our unpublished data showed that eugenol is not capable to induce sedation in carps. Cardiovascular assessments indicated that when largemouth bass, *Micropterus salmoides* exposed to clove oil of any concentration, showed an increase in cardiac output and heart rate following an initial bradycardia (Cooke *et al*., 2004). Hoskonen and Pirhonen (2004) reported the disturbance in color-changing ability of four different salmon fish including rainbow trout, *Oncorhynchus mykiss* when exposed to sedation concentrations of clove oil for three hours.

The result of this research reveals acceptable

Table 3. The numbers of PLs of *F. indicus* (n = 500) sitting down on the bottom of their containers during 24h exposure to different concentrations of eugenol in the sedation test

Concentration of eugenol (mg/L)	Time (h)						
	0	4	8	12	16	20	24
3.7	0	67	83	17	0	0	0
2.5	0	133	17	33	17	0	0
1.3	0	100	0	0	0	0	0
control	0	0	0	0	0	0	0

Table 4. The range of Reactions* of the PLs of *F. indicus* to indirect stimuli (knocking on the containers) during exposure to different concentrations of eugenol in sedation test

Concentration of eugenol (mg/L)	Reactions of PLs at various times (h)						
	0h	4h	8h	12h	16h	20h	24h
3.7	1	3	4	4	4	4	4
2.5	1	2	2	3	3	4	4
1.3	1	2	2	3	3	3	3
control	1	1	1	1	1	1	1

* Notes in the reactions:
4= Low reaction (PLs do not react even after 4 knocks playing on the container)
3= Delay reaction (PLs react after playing 3 to 4 knocks on the container)
2 = Delay reaction (PLs react after playing1 to 2 knocks on the container)
1 = Fast reaction to indirect stimuli (the PLs react as the pointer was in contact with the container)

Table 5. Concentrations of Ammonia -N and dissolved oxygen in the exposure seawater of PLs of *F. indicus* during sedation experiment. Data are the mean ± SD of three samples

Concentrations of eugenol (mg/L)	Ammonia-N (mg/L)	Dissolved oxygen (mg/L)
3.7	0.017 ± 0.004	4.20 ± 0.11
2.5	0.016 ± 0.007	3.57 ± 0.50
1.3	0.016 ± 0.004	2.69 ± 0.44
Control	0.016 ± 0.005	1.88 ± 0.00

sedation effect of eugenol in PLs of *F. indicus*. Although previous results derived from Coyle *et al*. (2005) works have also shown that clove oil had proper action in induction of sedation in the freshwater shrimp, but in this experiment PLs showed bottom sitting as they exposed to sedation concentrations of eugenol.

Apart from reduction in activity, low doses of anesthetics are also used to reduce metabolic rate of aquatics during transportation. This may reduce physiological stress, oxygen consumption and CO_2 and ammonia production (Wedemeyer, 1996; Ross and Ross, 1999) and thus decrease mortalities during and after transportation. In this research, measurement of dissolved oxygen and ammonia contents of the exposure seawater during 12 hours induction of sedation showed no statistical differences between ammonia-N contents of seawater in the experimental and control containers. However, statistical differences were found between oxygen contents of seawater in the experimental and control containers and also within experimental containers presented various concentrations of eugenol. No report was found to reveal the effect of sedative on metabolic rate of shrimp. However, Wedemeyer (1996) reported that typical oxygen consumption rates of spring Chinook salmon, *Oncorhynchus tshawytscha* smolts are 210 mg kg/1h/1 in untreated transportation tank water and 190 mg kg/1h/1 when 10 mg/L MS-222 is added. Kaiser and Vine (1998) have also observed that 2-phenoxyethanol did not affect the oxygen consumption or ammonia production of goldfish, *Carassius auratus* during transport; on the other hand Teo and Chen (1993) have showed that it suppressed oxygen consumption rates of guppy, *Poecilia reticulata*, in simulated transportation experiment. In simulated air transport of platy fish, *Xiphophorus maculates*, 2-phenoxyethanol and quinaldine sulphate were efficient in decreasing the excretion of CO_2 and ammonia, MS-222 reduced ammonia but not CO_2 production and metomidate had no effect on excretion of metabolic wastes (Guo *et al*., 1995). Pavlidis *et al*. (2003) observed that the use of anesthetic (ethynelglycol-monophenylether) during simulated transportation of red porgy, *Pagrus pagrus* fry had no significant effect on CO_2, NH_3 and NH_4 concentrations of exposure water. The results showed by Hoskonen and Pirhonen (2004) experiment indicate wide variation in oxygen consumption between six species of fish in response to low concentrations of clove oil during extended exposure. These examples indicate that different anesthetics

may differ in their efficiency and it may also indicate the presence of interfish-species variability in metabolic rates during exposure to low sedation concentration of anesthetics.

In the present study, bottom sitting appeared in experimental animals during sedation. This was recorded for all exposure concentrations used for induction of sedation (Table 3). In our previous study regarding to the sedation effects of eugenol on juvenile of *F. indicus* bottom sitting was observed in animals exposed to eugenol at 67% of the estimated 24hLC50, but not when eugenol was introduced as safe sedation concentration, i.e. 40% of the estimated 24hLC50. In the present study, however, bottom sitting was observed even at concentration equal value to 24% of the estimated 24hLC50. This indicates that PLs of *F. indicus* are more sensitive to eugenol. Bottom sitting by the use of low concentrations of eugenol or any other sedatives that do not harm the experimental animal has not been reported elsewhere for shrimp.

The ideal level of sedation for fish transport is referred to as deep sedation and includes loss of reactivity to external stimuli, decrease in metabolic rate, but maintenance of equilibrium (McFarland, 1959). This level of anesthesia is consistent with stage II of anesthesia as described by Summerfelt and Smith (1990). Recently, Hoskonen and Pirhonen (2004) stated that only a relatively small increase in clove oil concentration above the sedation concentration may cause the fish to lose equilibrium. They reported that the safety margin of clove oil may be too small for transport purposes, at least for some species of fish. However, as there were no mortality in the PLs of *F. indicus* exposed to sedation concentrations of eugenol during 24 hours test, loss of equilibrium during sedation of pelagic aquatics has not been defined where it supposed that for shrimp as a benthic animal, it may be a regular phenomena.

Like other medicines, in view of the practical use of eugenol as sedative for PLs of *F. indicus*, a toxicity study regulated mitigation to confirm the safety margin of its usage (Rombough, 2007). The results of toxicity test of eugenol on PLs of *F. indicus* showed a regular negative correlation between concentration of eugenol and its exposure time (Table 2). A combination between the efficient concentration of eugenol for induction of sedation during 12 hours transportation and 12hLC50 of eugenol on *F. indicus* showed that 1.3 mg/L of eugenol could be more applicable as the proper sedative concentration. According to the results, this concentration has lower effective time on bottom sitting of PLs during sedation and higher safety margin with compare to the minimum confidence limit of 12hLC50.

In conclusion, results indicate that eugenol is effective as a sedative for transportation of PLs of *F. indicus*. However, the concentration inducing sedation can cause an effect known as bottom sitting. Therefore, the use of eugenol to sedate PLs of *F. indicus* during transport should be considered with care. Additional research is needed to determine the basic effect of eugenol on bottom sitting of PLs of *F.indicus* during sedation and to find out if it can be prevented by other trials.

Acknowledgements

Many thanks to Mr. Bahram Ghanadian for helping us to conduct this research in his well developed shrimp hatchery located in Delvar, Bosher Province, IR Iran. The attempts of Deputy Dean and staff of SRC of Persian Gulf for determination of water quality parameters also greatly appreciated.

References

Akbari, S. and Khajavi, A. 2004. Comparison between MS-222 and eugenol as sedative and anesthetic agent in shrimp, *Penaeus indicus*. In: 7th Asian Fisheries Forum, 30 Nov. - 4 Dec. Penang, Malaysia, 144 pp.

Akbari, S. and Shariff, M. 2003. Stress response of red snapper, *Lutjanus argentimaculatus* exposed to sublethal concentrations of crude oil. Arhc. Razi Inst., 54: 49-64.

Akbari, S. and Tokhm Afshan, M. 2001. Study of effective factors on qualitative standards of *Penaeus indicus* post larvae. Pajouhesh-va-Sazandegi. 52: 88-91.

Akbari, S., Law, A.T. and Shariff, M. 2004. Toxicity of water soluble fraction of crude oil to fish, *Lutjanus argentimaculatus* and shrimp, *Penaeus monodon*. Iranian J. of Sci. Technol., 28A: 169-175.

APHA, 1992. Standard Methods for the Examination of Water and Wastewater. M.A.H. Franson, A.E. Greenberg, L.S. Clesveri, A.D. Eaton (Eds.), Washington: 4-81.

Cooke, S.J., Cory, D., Suski, K.G., Ostrand, Tufts B.L. and Wahl, D.H. 2004. Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting largemouth bass (*Micropterus salmoides*). Aquaculture, 239: 509-529.

Cordova, M.S. and Braun, C.B. 2007. The use of anesthesia during evoked potential eudiometry in goldfish (*Carassius auratus*). Brain Res., 1153: 78-83.

Coyle, S.D., Dasgupta, S., Tidwell, T.H., Beavers, T., Bright, L.A. and Yasharian, D.K. 2005. Comparative efficacy of anesthetics for the freshwater prawn *Macrobrachiurn rosenbergii.* J. World Aquaculture Soc. 36: 282–290.

Gowen, R.J., Tett, P., Kennington, K., Mills, D.K., Shammon, T.M., Stewart, B.M., Greenwood, N., Flanagan, C., Devlin, M. and Wither, A. 2007. The Irish Sea: Is it eutrophic estuarine, Coastal and Shelf Sci., 76: 239-254.

Guo, F.C., Teo, L.H. and Chen, T.W. 1995. Effects of anaesthetics on the water parameters in a simulated transport experiment of platyfish, *Xiphophorus maculatus* (Guenther). Aquaculture Res. 26: 265-271.

Harper, C. 2003. Status of clove oil and eugenol for anesthesia of fish. Aquaculture Magazine, 29: 41–42.

Hoskonen, P. and Pirhonen, J. 2004. Temperature effects on anaesthesia with clove oil in six temperate-zone fishes. J. Fish Biol., 64: 1136-1142.

Jolly, D.W., Mawdesley-Thomas, L.E. and Bucke, D. 1972.

Anesthesia of fish.Vet. Rec., 91: 424-426.

Kaiser, H. and Vine, N. 1998. The effect of 2-phenoxyethanol and transport packing density on the post-transport survival rate and metabolic activity in the goldfish, *Carassius auratus*. Aquarium Sci. Cons., 2: 1-7.

Li, T. and Brouwer, M. 2007. Hypoxia-inducible factor, gsHIF, of the grass shrimp *Palaemonetes pugio*: Molecular characterization and response to hypoxia. Comp. Biochem. Physiol., 147 (B): 11-19.

McFarland, W.N. 1959. A study of the effects of anesthetics on the behaviour and physiology of fishes. Publication of the Institute of Marine Sciences, University of Texas, 6: 23-55.

Palic, D., Herolt, D.M., Andreasen, C.B., Menzel, B.W. and Roth, J.A. 2006. Anesthetic efficacy of tricaine methanesulfonate, metomidate and eugenol: Effects on plasma cortisol concentration and neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820). Aquaculture, 254: 675-685.

Parsons, T.R., Maita, Y. and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York, 173 pp.

Pavlidis, M., Angellotti, L., Papandroulakis, N. and Divanach, P. 2003. Evaluation of transportation procedures on water quality and fry performance in red porgy (*Pagrus pagrus)* fry. Aquaculture, 218: 187-202.

Rombough, P.J. 2007. Ontogenetic changes in the toxicity and efficacy of the anaesthetic MS222 (tricaine methanesulfonate) in zebrafish (*Danio rerio*) larvae. Comp. Biochem. Physiol., 148: 463-469.

Ross, L.G. and Ross, B. 1999. Anaesthetic and sedative techniques for aquatic animals. Second Edition, Blackwell Science Ltd, Oxford, UK, 176 pp.

Spraque, J.B. 1990. Aquatic toxicology. C.B. Schreck and P.B. Moyle (Eds.), Methods for Fish Biology. Am. Fish. Soc., Bethesda, Maryland: 491-528.

Summerfelt, R.C. and Smith, L.S. 1990. Anesthaesia, surgery, and related techniques. C.B. Schreck and P.B. Moyle (Eds.), Methods for Fish Biology. Am. Fish. Soc., Bethesda, Maryland: 213–272.

Teo, C.H. and Chen, T.W. 1993. A study of metabolic rates of *Poecilia reticulata* Peters under different conditions. Aquaculture and Fisheries Manage., 24: 109-117.

Wedemeyer, G.A. 1996. Transportation and handling. W. Pennell and B.A. Barton (Eds.), Principles of Salmonid Culture, Elsevier, Amsterdam, and the Netherlands: 727-758.