PANAMJAS
Pan-American Journal of Aquatic Sciences

Evaluation of natural extracts with anesthetic properties in juveniles *Macrobrachium tenellum*

MARTÍN ALONSO ARÉCHIGA PALOMERA[1], OSCAR BASILIO DEL RIO ZARAGOZA[2], SAÚL ROGELIO GUERRERO GALVÁN[1*] & FERNANDO VEGA VILLASANTE[1].

[1] *Laboratorio de Calidad de Agua y Acuicultura Experimental. Centro de Investigaciones Costeras. Universidad de Guadalajara. Av. Universidad 203, Delegación Ixtapa, 48280 Puerto Vallarta, Jalisco, México.*
[2] *Centro de Investigaciones Oceanológicas. Universidad Autónoma de Baja California. Carr, Ensenada-Tijuana no. 3917, Fraccionamiento Playitas, 22860, Ensenada, Baja California, México.*

*Corresponding author: guerrero_saul@yahoo.com.mx

Abstract: Several aquaculture activities stresses organisms; anesthesia during this practices could reduce it. The anesthetic effect of extracts of, *Passiflora incarnata*, *Valeriana officinalis*, *Syzygium aromaticum* and menthol, were tested on juvenile prawns as an alternative to reduce stress. Only the last two showed effect.

Key Words: clove, menthol, aquaculture, prawns

Resumen Evaluación de extractos naturales con propiedades anestésicas en juveniles de *Macrobrachium tenellum*. Algunas actividades estresan los organismos. En este estudio se evaluó el efecto anestésico de extractos naturales, *Passiflora incarnata*, *Valeriana officinalis*, *Syzygium aromaticum* y mentol en langostinos juveniles como una alternativa para disminuir el estrés. Solamente los últimos dos mostraron efecto.

Palabras clave: clavo, mentol, acuicultura, langostinos

Aquaculture activities such as capture, handling, storing and transportation cause stress and physiological alterations in organisms (Barton *et al*. 2000, Davis 2004, Jentoft *et al*. 2005). Stress has a negative impact on animals, which includes reduction of immunocompetence, increased susceptibility to diseases, reduction of roe quality, growth (Pickering 1981); skin darkening and decrease on market value (VanderSalm *et al*. 2004). The river prawn *Macrobrachium tenellum* is distributed from Mulege in Baja California, Mexico, to the Chira river in northern of Peru (Holthuis 1952). It is a species of economic importance because it is subject of artisanal fishing in coastal regions of Mexico, El Salvador and Guatemala, either for self-consumption or sale (Cabrera 1983). *M. tenellum* is considered an interesting potential aquaculture prawn because it is not aggressive and could be cultured at high densities (García-Guerrero et al. 2013).

Freshwater prawn *Macrobrachium rosenbergii* 0.3-0.6 g. (D'Abramo *et al*. 1995) are transported as juvenile from the nursery to the production ponds, the stress during transportation is often the reason of mortality (Coyle *et al*. 2001). Recently, the attention has been focused into minimizing the stress through various techniques. Nutraceuticals and high protein diets can mitigate stress in farming conditions; vitamin C also reduces stress from physical injuries in *M. rosenbergii* (Manush *et al*. 2005). Anesthetics are widely use to minimize fish handling stress (Pal & Choudhary 2003) and crustaceans (Venkateshwarlu & Pal 2003). In some suggested strategies to reduce physiological alterations in shrimp resulted from stress during handling, anesthetics are of great significance. At present, the anesthetics approved by the U.S. Food and Drug Administration (FDA) are: tricaine methanesulfonate (MS-222) and carbon dioxide (CO_2) (Schnick *et al*. 1986). From the anesthetics

approved by the FDA for aquaculture, the tricaine methanesulfonate (MS-222) is not an effective one for many crustaceans (Coyle *et al*. 2004). Its use causes several risks and additionally has a setting time of 21 days before the product could be consumed (Smith *et al*. 1999). There are no reports of the use of carbon dioxide on crustaceans for anesthetic purposes. Cold anesthetic with refrigerated sawdust has been reported efficient for short-term transportation (Salin 2005). There is a growing interest in the use of natural components with anesthetic effects in aquaculture, such as eugenol/clove oil (Anderson *et al.* 1997, Stone & Tostin 1999). "Aqui S ®" is a commercial anesthetic that has been developed for aquaculture use (Stehly & Gingerich, 1999, Davidson *et al*. 2000); Clove oil and Aqui S ® induce anesthesia on low concentrations, unlike quinaldine which has been used as anesthetic on *M. rosenbergii* (Coyle *et al*. 2005). This study represents the first attempt to evaluate the anesthetic effect of natural extracts of Passion Flower (*Passiflora incarnata*), clove (*Syzygium aromaticum*), valerian (*Valeriana officinalis*), and menthol, on juvenile prawns *Macrobrachium tenellum*, in order to reduce stress in organisms during transportation to culture facilities or handling laboratory procedures.

Bioassays were done at the Water Quality and Experimental Aquaculture Laboratory of the Coastal Research Center of the University of Guadalajara 20°42'19" N 105°13'16" W, 10 meters above mean sea level, in Puerto Vallarta, Jalisco, Mexico. Glass fish tanks with (Elite ®) cascade filters were used. Temperature was a continual 28°C, by the use of (Sunny ®) thermostats. Water was obtained from the municipal system and previously at rest to eliminate excess chlorine. Juvenile prawns *M. tenellum* were collected in "El Zarco" riverbed stream, in the municipality of Puerto Vallarta, Jalisco. All organisms were acclimatized for seven days before experimentation. The mean size and weight of organisms were 46 ± 4 mm and 1.1 ± 0.3 g, respectively. *P. incarnata* (flowers 50 g) and *V. officinalis* (roots 50 g) infusions were done on 300 mL of distilled water. Clove oil (Eugenol 82 – 87 %) ®, was diluted at 1:2 in ethanol 70 % and the crystallized menthol was dissolved in ethanol 70 % before to be added to experimental tanks. Ten juvenile prawns *M. tenellum* were set in each fish tank and exposed to three concentrations (300, 600 and 900 mg/L) of *P. incarnate*, *V. officinalis* and menthol. The stock solution of Eugenol (ethanolic) was used to obtain concentrations 300, 600 and 900 ml/L of Eugenol. Also, experimental control treatments were made without extracts, and with 600, 1200 and 1800µl/L of ethanol 70 %. All treatments were done by triplicate. Relative anesthesia of prawns was based in the anesthetic stadiums described by Coyle *et al*. (2005). A value from one to four was given to the anesthetic stadiums. Number 1 shows a fast response to tactile stimuli because prawn is not yet affected by anesthetic and shows a normal escape response to tactile stimuli. Number 2 shows that the prawn has a partial loss of balance but a reaction is still observed to tactile stimuli and returns to position when is turned upside, also, a slow pleopods movement can be seen. Number 3 is for the time when the prawn losses balance; it has no reaction to tactile stimuli and presents a loss of pleopods movement. Number 4 shows euthanasia. Prawns that showed stadium 3 were taken into ventilated anesthesia-free tanks for their recovery. Anesthetic induction and recuperation were evaluated registering the required time to reach stadium 3 and the return to stadium 1 of each organism. The effect of the different doses of natural extracts with anesthetic effect were compared among each other with a one-way variance analysis on the values of induction and recovery time from anesthesia, previous normality tests (Kolmogorov-Smirnov; $\alpha = 0.05$) and homoscedasticity (Bartlett; $\alpha = 0.05$) for each case, all this through the SigmaStat V3.1 (Systat, 2007), Statistical Software. Significant differences among treatments were determined by multiple comparisons Duncan test ($\alpha = 0.05$). (Table I).

Time needed to induce deep anesthesia (stadium 3), recovery time and survival of juvenile prawns *M. tenellum* exposed to different solutions (ethanol, clove and menthol) are shown in Table I. There were no significant differences presented (Duncan test $p(\alpha)>0.05$) for the exposed prawns to clove treatment of 300 µL/L, regarding deep anesthesia induction time (17 ± 7 minutes), clove treatment of 600 µL/L (12 ± 4 minutes of induction), but, there was a significant difference with the clove treatment of 900 µL/L regarding that of 600 µL/L, with a lower induction time of 8 ± 3 minutes (Table I). On the prawns exposed to menthol, the only treatment that took the prawns to a deep anesthesia induction, was the one of menthol to 900 mg/L, with an induction time more than 120 minutes which is significantly higher regarding clove treatments (Duncan $p(\alpha)<0.05$). Anesthetic recovery time were similar (Duncan $p(\alpha)>0.05$) in all clove concentrations, while recovery time for menthol

Table I. Time required for inducing deep anesthesia, recovery time and survival of juvenile prawns *Macrobrachium tenellum* exposed to different solutions (ethanol, clove and menthol). Values represent the mean ± SD of three replicas. Superscripts show statistically significant differences between treatments ($p(\alpha) < 0.05$) different superscripts show differences. No significant differences ($p(\alpha) > 0.05$) were found in survival. The sign "-" indicates that no anesthetic induction was reported.

Treatment	Dose	Induction (min)	Recuperation (min)	Survival (%)
No extract	-	-	-	100
Ethanol 70%	300-1,800 µL/Ll	-	-	100
Clove	300 µL/L	17 ± 5^{b}	20 ± 6^{d}	100
Clove	600 µL/L	12 ± 4^{b}	27 ± 3^{d}	100
Clove	900 µL/L	8 ± 3^{c}	34 ± 16^{d}	100
Menthol	900 mg/L	$>120^{a}$	53 ± 19^{e}	100

treatments 900 mg/L were significantly higher regarding clove treatments (Duncan $p(\alpha)<0.05$).

There was only 3.3% mortality represented in the experimental population with the menthol treatment of 600 mg/L, even though it was not a significant one regarding to other treatments, determined with Duncan tests ($p(\alpha)>0.05$). The evolution of different anesthetic stadiums at each treatment of experimental population were determined measuring proportion of population in each anesthetic stadium every five minutes till all population were in stadium 3 (Tables II and III). Percentages of anesthetic stadiums and exposure time to solutions of clove and menthol to *M. tenellum* are show in Tables II and III, respectively. Clove, and menthol treatments reach stadium 2 and 3, at different percentages and times. There was a time reduction for the experimental population to reach maximum anesthetic stadium with clove treatments: 300 µL/L, 100% of the experimental population reached maximum anesthetic stadium in 25 minutes, 600 µL/L, 100% of the experimental population reached maximum anesthetic stadium in 20 minutes, 900 µL/L, 100% of the experimental population reached maximum anesthetic stadium in 15 minutes. The same phenomenon was observed in menthol treatments, to higher concentration less time was needed for the organisms to reach maximum anesthetic stadium, the only difference in this case was that not all experimental population reaches the maximum anesthetic stadium and time required to reach anesthetic stadiums is longer (Table III). All experimental population on Passion Flower, valerian and experimental control treatments, stayed in stadium 1 at all the concentrations tested (from 300 to 900µL/L), during the 120 minutes of the bioassays that is, no anesthetic effect was observed (Table III).

In this study, experiments of anesthesia with Passion Flower, and Valerian did not induce anesthesia in prawns at concentrations used. However, this is the first study where Passion Flower and valerian are used as possible anesthetic candidates. Passion Flower has been widely used in traditional medicine (Patel *et al*. 2008); because it has alkaloid compounds (Dhawan *et al*. 2004). Expected effects in this study, could have been similar to those that have given potential effects for some neurological diseases such as anxiety, insomnia and hyperactive disorder (Patel *et al*. 2008). There has been significant sedative activity credited on valerian used for mice (Murti *et al*. 2011). However, results in this work did not show any anesthetic effect in prawns *M. tenellum*. The result showed in this study suggests the use of clove extract (eugenol) for inducing deep anesthesia in prawns *M. tenellum*, because anesthetic induction times to induce deep anesthesia are low (8 to 17 minutes) as well recuperation time (20 to 34 minutes) in all experimental population treated with clove oil, in all concentrations used. The anesthetic effect matches the one reported for fish, such as the rainbow trout *Oncorhynchus mykiss* (Keene *et al*. 1998). Clove oil, specifically eugenol, is easy to use and could be use in lower concentrations than other local anesthetics (Keene *et al*. 1998) and is metabolized and excreted rapidly so there is no need for a period for excretion (Wagner *et al*. 2002). The use of clove oil in crustaceans as the one of Ozeki (1975), which reported that eugenol is the active ingredient for clove oil, and that it could be an effective anesthetic for prawn *Procambarus clarkii* in doses of 200-1000 ppm. Morgan *et al.* (2001) evaluated clove oil for anesthetic use in three Pacific Coast crayfish species (*Cancer magister, Hemigrapsus oregonensis* and *Pugettia producta*) and discovered great differences in concentrations used to induce anesthesia in different species. These

differences in efficiency of anesthetics could be because of the specificity of chemical receptors. Saydmohammed & Pal (2009), evaluated a mix of anesthetics with different relative densities (eugenol and menthol). The critical dose of the anesthetic formula that was found was 200µL/L, in 30 minutes. In comparison to this study, deep anesthesia was reached in 17 minutes with 300µL/L in *M. tenellum*. Recovery for prawns, once removed from anesthetic exposition, showed a better margin of safety. Brett (1964) y Kutty (1968) mentioned, in his studies with fish that due to handling in presence of anesthetic formula, a reduction of oxygen consumption with an increase of anesthetic doses show low energetic usage to face stress. Diverse motor neurons are involved in crustacean movement, depending on the type of locomotion (phasic neurons with fast response movements and tonic neurons for slow and gradual response movements) depending on activity levels (Millar & Atwood 2004). Saydmohammed & Pal (2009) concluded that a sole anesthetic could be inefficient for anesthetic purposes in different receptors. Therefore, the anesthetic formula (eugenol-menthol) of Saydmohammed & Pal (2009), has different levels of anesthesia that could have influenced in the different synaptic boutons of crayfish to suppress activity levels that was evident in the decrease of the oxygen consumption rate. Culloty & Mulcahy (1992), show that menthol induces half anesthesia in oyster, *Ostreaadulis*, when applied to in a concentration of 2%. Saydmohammed & Pal (2009), reported that menthol applied to *M. rosenbergii*, induces a significantly higher anesthetic time induction to that of the clove oil, eugenol. This matches the results of this study, where eugenol induces deep anesthesia faster than menthol. An ideal anesthetic should rapidly induce anesthesia with minimum hyperactivity or stress. It should be easily given and should keep the animal in the desired stage. When anesthetic is cleared from the animal, recovery should be fast. The anesthetic should be efficient on lower doses, and toxic dose should largely exceed effective dose so there is a large safety margin (Coyle *et al*. 2004). Strictly speaking, clove oil, used in this study, as anesthesia for juvenile prawn *M. tenellum,* is found to be better suited than menthol, as for its rapid anesthetic induction as for its recovery. Evaluation of ethanol anesthetic effects on crustaceans constitutes the first report. Even though, ethanol effect was not observed with the concentrations used in this study. In the contrary to results in this study, ethanol has been effective for other invertebrates at higher concentrations, for example, applied to squid *Sepia* sp, in a solution of 1.5 to 3% (Harms *et al.* 2006). Similarly, anesthetic for abalone, ethanol at 3%, anesthetic for gastropods, ethanol at 5% (Gunkel & Lewbart 2008); anesthetic for earthworms *Lumbricus terrestris*, ethanol at 5% (Marks & Cooper 1977); anesthetic for turbellaria at 10% (Pennak, 1978). There is also a report on the combination of ethanol and menthol (Listerine ®) to 10% as anesthetic for gastropods (Woodall *et al*. 2003). The possibility of anesthetic effect on higher doses of ethanol from those applied in this study to juvenile prawns *M. tenellum* is not ruled out. Could be interesting to analyze higher concentrations of ethanol, bioassays with 3% ethanol show euthanasia of prawn *M. tenellum* (results not shown), as reported by Murray (2006), who uses 10% ethanol to test euthanasia on cephalopods. In the bioassays of this study, there was mortality registered in the menthol treatment at 600 µL/L that represented 3.6% of that experimental population, but it was not a significant one ($p(\alpha)>0.05$) in reference to other treatments (Table I). Coyle *et al*. (2005) reported mortality in *M. rosenbergii* in 100, 200 y 300 mg/L concentrations of quinaldine and Aqui-S^{TM} (commercial product of clove oil). In contrast with these results, Saydmohammed & Pal (2009), reported mortality when using eugenol and menthol (100, 200, 400 y 800 µL/L) as anesthetic for prawn *M. rosenbergii.* Clove oil components and menthol are efficient in lower doses and show a large safety margin to be used as anesthetics, furthermore they are ideal anesthetics because they are able to induce anesthesia rapidly with minimal hyperactivity and are easily applied to organisms.

Clove oil is the natural compound that turned out to be more efficient to induce anesthesia, as well as for recovery of juvenile prawns *Macrobrachium tenellum*. This study provides important knowledge that will help reduce stress and mortality of *M. tenellum* caused by capture, handling, storing and transportation activities. Based on the results obtained in this study and compared with those found in other crustacean species, including some very close in their phylogeny, it is evident that concentrations of anesthetics are particular to each species. Anesthetic concentrations established for a particular organism will not necessarily be useful or safe for other species.

Table II. Percentage in the stadium and at the time of the experimental population of shrimp *Macrobrachium tenellum* exposed to different anesthetic solutions, where: E1: the prawns have pleopods flicks with a response to a tactile stimulus; E2: partial loss of balance and response to tactile stimulation with slow movements, pleopods; E3: Total loss of balance and response to tactile stimulation with very slow movements or zero pleopods. Bioassays were 120 minutes duration.

Treatment	Dose	5 min	10 min	15 min	20 min	25 min
		E1-E2-E3	E1-E2-E3	E1-E2-E3	E1-E2-E3	E1-E2-E3
Control	0	100-0-0	100-0-0	100-0-0	100-0-0	100-0-0
Ethanol	300-1,800 µL/L	100-0-0	100-0-0	100-0-0	100-0-0	100-0-0
Clove	300 µL/L	10-90-0	0-80-20	0-37-63	0-13-87	0-0-100
Clove	600 µL/L	0-90-10	0-50-50	0-7-93	0-0-100	- - -
Clove	900 µL/L	7-53-40	0-20-80	0-0-100	- - -	- - -

(-) All individuals peaked anesthetic stadium

Table III. Percentage in the stadium and at the time of the experimental population of shrimp *Macrobrachium tenellum* exposed to different anesthetic solutions, where: E1: the prawns have pleopods flicks with a response to a tactile stimulus; E2: partial loss of balance and response to tactile stimulation with slow movements, pleopods; E3: Total loss of balance and response to tactile stimulation with very slow movements or zero pleopods. Bioassays were 120 minutes duration.

Treatment	Dose	90 min	100 min	110 min	120 min
		E1-E2-E3	E1-E2-E3	E1-E2-E3	E1-E2-E3
		90 min	100 min	110 min	120 min
Menthol	300 mg/L	90-10-0	60-40-0	17-83-0	3-97-0
Menthol	600 mg/L	50-50-0	43-57-0	37-63-0	27-57-16
Menthol	900 mg/L	13-74-13	7-66-27	0-50-50	0-23-77
Passion Flower	300-900 mg/L	100-0-0	100-0-0	100-0-0	100-0-0
Valerian	300-900 mg/L	100-0-0	100-0-0	100-0-0	100-0-0

References

Anderson, W. G., McKinley, R. S. & Colavecchia, M. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. **North American Journal of Fisheries Management**, 17: 301–307.

Barton, B. A., Bollig, H., Hauskins, B. L. & Jansen, C.R. 2000. Juvenile pallid (*Scaphirhynchus albus*) and hybrid pallid × shovelnose (*S. albus* × *platorynchus*) sturgeons exhibit low physiological responses to acute handling and severe confinement. **Comparative Biochemistry and Physiology**, 126(1): 125–134.

Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. **Journal of the Fisheries Research Board of Canada**, 21(5): 1183–1226.

Cabrera, P. J. 1983. Carácter práctico para diferenciación de sexos en *Macrobrachium tenellum* (Crustacea: Decapoda: Natatia). **Revista de Biología Tropical**, 31: 159-160.

Coyle, S. D., Durborow, R.M. & Tidwell, J. H. 2004. Anesthetics in Aquaculture. **Southern Regional Aquaculture Center (SRAC),** 3900: 1–6.

Coyle, S. D., J. H. Tidwell. & VanAmum, A. 2001. The effect of biomass density on transport survival of juvenile freshwater prawn, *Macrobrachium rosenbergii*. **Journal of Applied Aquaculture**, 11(3): 57-63.

Coyle, S. D., Dasgupta, S., Tidwell, J. H., Beavers, T., Bright, L.A. & Yasharan, D. K. 2005. Comparative efficacy of anesthetics for the freshwater prawn *Macrobrachium rosenbergii*. **Journal of World Aquaculture Society**, 36(3): 282–290.

Culloty, S. C. & Mulcahy M. F. 1992. An evaluation of anesthetics for *Ostrea edulis* (L.). **Aquaculture**, 107(2-3): 249-252.

D'Abramo, L. R., Daniels, W. H; Fondren, M. W. & Brunson, M. W. 1995. **Management practices for culture of freshwater shrimp (*Macrobrachium rosenbergii*) in temperate**

climates. Bulletin 1030. Mississippi Agricultural Forestry Experimental Station, Mississippi State University, Mississippi, USA. Econometric Software Inc. 1998. LIMDEP, version 7, Plainview, New York.

Davidson, G. W., Davie, P. S., Young, G. & Fowler, R. T. 2000. Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S. **Journal of World Aquaculture Society**, 31(1): 105–114.

Davis, K. B. 2004. Temperature affects physiological stress responses to acute confinement in sunshine bass (*Morone chrysops* × *Morone saxatilis*). ***Comparative Biochemistry and Physiology***, 139(4): 433–440.

Dhawan, K., Dhawan, S. & Sharma, A. 2004. *Passiflora*: A review update. **Journal Ethnopharmacol**, 94(1): 1-23.

García-Guerrero, M., Becerril-Morales F., Vega-Villasante F & Espinosa-Chuarand L. 2013. Los langostinos del género Macrobrachium con importancia económica y pesquera en América Latina: conocimiento actual, rol ecológico y conservación. **Latin American Journal of Aquatic Research**, 41: 651-675.

Gunkel, C. & Lewbart, G. A. 2008. Anesthesia and analgesia of invertebrates. Pp. 535-536. *In*: Fish, R., Danneman., Brown, M., Danneman, P.J. & Karas, A.Z. (Eds.). **Anesthesia and Analgesia in Laboratory Animals**. Elsevier inc, The United States of America, 672 p.

Harms, C. A., Lewbart, G. A. & McAlarney R. 2006. Surgical excision of mycotic (*Cladosporium* sp.) granulomas from the mantle of a cuttlefish (*Sepia officinalis*). **The *Journal of Zoo* and *Wildlife Medicine,*** 37(4): 524-530.

Holthuis, L. B., 1952. A general revision of the Palaemonidae (Crustacea: Decapoda: Natatia) of the Americas. II. The subfamily Palaemonidae. **Allan Hancock Foundation Occasional Papers**, 12: 11-132.

Jentoft, S., Aastveit, A. H., Torjesen, P. A. & Andersen, Ø. 2005. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). **Comparative Biochemistry and Physiology**, 141(3): 353–358.

Keene, J. L., Noakes, D. L. G; Moccia, R. & Soto, C. G. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). **Aquaculture Research**, 29(2): 89–101.

Kutty, M. N. 1968. Respiratory quotient in gold fish and rainbow trout. **Journal of the Fisheries Research Board of Canada**, 25(8): 1689-1728.

Manush, S. M., Pal, A. K., Das, T. & Mukherjee, S.C. 2005. Dietary high protein and vitamin C influence in mitigating stress due to chelate claw ablation in *Macrobrachium rosenbergii* males. **Comparative Biochemistry and Physiology**, A142: 10–18.

Marks, D.H., & Cooper, E.L. (1977). *Aeromonas hydrophila* in the coelomic cavity of the earthworms *Lumbricus terrestris* and Eisenia foetida. **Journal of Invertebrate Pathology**, 29 (3): 382-383.

Millar, A. G. & Atwood, H. L. 2004. Crustacean phasic and tonic motor neurons. **Integrative & Comparative Biology**, 44(1): 4-13.

Morgan, J., Cargill, C. & Groot, E. 2001. The efficacy of clove oil as an anesthetic for decapod crustaceans. **Bulletin of the Aquaculture Association of Canada***,* 101(3): 27-31.

Murray, M. J. 2006. Euthanasia. P.p 303-304. *In*: Lewbart, G.A. (ed.). **Invertebrate Medicine**. Ames IA: Blackwell Publishing, 550 p.

Murti, K; Kaushik, M; Sangwan, Y. & Kaushik, A. 2011. Pharmacological properties of *Valeriana officinalis*. A review**.** **Pharmacology online**, 3: 641-646.

Ozeki, M. 1975. The effect of eugenol on the nerve and muscle in crayfish. **Comparative Biochemical Physiology***,* 50(2): 183-191.

Pal, A. K. & Choudhary, M. 2003. Anesthetics and other antistress compounds in handling and transportation of live finfish. Pp. 220-232. *In*: Bright Singh, I.S., Pai Somnath, S., Rosamma, P., Mohandas, A. (Eds.). **Aquaculture Medicine**. Cochin University of Science and Technology, 336 pp.

Patel, S. S., Mohamed Saleem, T.S., Ravi, V., Shrestha, B., Verma, N.K. & Gauthaman, K. (2008). *Passiflora incarnata* Linn: A phytopharmacological review. **International Journal of Green Pharmacy**, 3(4): 277-280.

Pennak, R. W. 1978. *Turbellaria* (flatworms). Pp. 114-141. *In*: Pennak RW (ed.). **Freshwater Invertebrates of the United States**. New York, NY, John Wiley and Sons, 664 p.

Pickering, A. D. (1981). Introduction: the concept of biological stress. Pp. 1-9. *In*: Pickering, A.D. (Ed.). **Stress and Fish London**. Academic Press, 367 p.

Salin, K. R. 2005. Live transportation of *Macrobrachium rosenbergii* (De Man) in chilled sawdust. **Aquaculture Research**, 36(3): 300–310.

Saydmohammed, M. & Pal, A. K. 2009. Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. **Aquaculture**, 298(1-2): 162–167.

Smith, D. A., Smith, S. A. & Holladay, M. 1999. Effects of previous exposure to tricane methane sulfonate one time anesthesia to hybrid tilapias. **Journal of Aquatic Animal Health**, 11(2): 183–186.

Schnick, R. A., Meyer, F. P. & Walsh, D. F. 1986. Status of fisheries chemicals in 1985. **Progressive Fish Culturist**, 48(1): 1-17.

Stehly, G.R. & Gingerich, W.H. 1999. Evaluation of AQUI-S (efficacy and minimum toxic concentration) as a fish anesthetic/sedative for public aquaculture in the United States. **Aquaculture Research**, 30(5): 365–372.

Stone, D. & Tostin, N. 1999. Clove bud oil a big yawn for silver perch: Fish. **NSW Mag. Spring**, 19.

Systat Software Inc. 2007. SigmaStat (data analysis sofware system), version 3.5. https://systatsoftware.com/

Vander Salm, A. L., Martínez, M., Flik, G. & Wendelaar, B. S. E. 2004. Effects of husbandry conditions on the skin colour and stress response of red porgy, *Pagrus pagrus,* **Aquaculture**, 241(1-4): 371–386.

Venkateshwarlu, G. & Pal, A. K. 2003. Anesthetics and other techniques for handling and transportation of live shellfish. Pp. 213-219. *In*: Bright Singh, I.S., Pai Somnath, S., Rosamma, P. & Mohandas, A. (Eds.). **Aquaculture Medicine**. Cochin University of Science and Technology, 336 pp.

Wagner, E., Arndt, R. & Hilton, B. 2002. Physiological stress responses, egg survival and sperm motility of rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide. **Aquaculture**, 211: 353– 366.

Woodall, A. J., Naruo, H. & Prince, D. J. 2003. Anesthetic treatment blocks synaptogenesis but not neuronal regeneration of cultured *Lymnaea neurons.* **Journal Neurophysiology**, 90(4): 2232-2239.

Received: May 2016
Accepted: August 2016
Published: October 2016