CC BY

Anesthetic effect of ethanol, MS222 and cold maintenance on freshwater crayfish (*Astacus leptodactylus)*

Seidgar M.[1*]; Jalili R.[1]; Nekoueifard A.[1]; Mirzargar, S.S.[2]; Abbaspour Anbi A.[1]; Alizadeh Osalou Zh.[1]

Received:September 2025 Accepted: December 2025

Abstract

Freshwater crayfish from the Aras Dam are considered one of the country's high-value fishery export resources. Freshwater crayfish are exported live and at low temperatures. Limited studies have been conducted on their welfare and the use of anesthetics in stressful situations such as handling, cooking, manipulation, sampling, and transportation. The present study was conducted to determine the effective dose and time of anesthesia induction and recovery time using different levels of MS-222 (levels 0, 50, 100, 200, 400, 800 and 1200 mg/L), ethanol (levels 0, 50, 100, 200, 400, 800, 1200 and 1600 mg/L) and cold (temperatures 4, 6, 8 and 25°C as control) in freshwater crayfish (*Astacus leptodactylus*). In this study, the duration of anesthesia induction and recovery time of freshwater crayfish were investigated with the aim of determining the appropriate anesthetic concentration for young crayfish weighing approximately 20±3 grams. The results of the present study showed that the use of high levels of MS-222 was not effective in anesthetizing freshwater crayfish. The use of ethanol at high levels (30%) was also able to anesthetize crayfish in a short time, but at low levels this process required a long time. The results also showed that a low temperature of 4°C had the shortest induction time and the fastest recovery from anesthesia compared to MS-222 and ethanol. Therefore, the use of ethanol due to high stress induction and MS-222 due to high price and low performance in freshwater crayfish anesthesia is not recommended.

Keywords: Anesthesia, MS-222, Ethanol, Cold, Freshwater crayfish

1-National Artemia Research Center, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Urmia, Iran
2-Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
*Corresponding author's Email: Seidgar2011@gmail.com

[Downloaded from injbir.com on 2026-02-04]

Introduction

Freshwater crayfish are used for human consumption, especially in European and American countries, ornamental aquariums, and scientific experiments (neurobiology). Given that crustaceans respond to signs of pain and stress, how they are treated during farming, catching, packaging, transportation, slaughtering, and cooking can affect their welfare and sale for human consumption (Ghanawi *et al.,* 2019). Considering the marketability, nutritional and export value of freshwater crayfish, as well as the goal of the Iranian Fisheries Science Research Institute and the Iranian Fisheries Organization to introduce Aras Dam crayfish as a new cultured species, it is essential to find solutions to reduce stress and handling them (Nekoueifard *et al.,* 2019; Ghorbanzadeh and Sedghpour, 2024). Because any handling and manipulation of crayfish leads to losses or a decrease in the quality of the exported product, the use of chemical or herbal substances is necessary to reduce stress during fishing, packaging, and transportation. When culturing and transporting aquatic animals, interventions such as bioassays, vaccinations, handling, and sorting are essential to achieve optimal yields. However, these processes can cause physical damage and physiological stress in aquatic animals, which increases susceptibility to infections caused by pathogens and ultimately mortality (Roth and Skra, 2021; Zhu *et al.,* 2023). Therefore, reducing injury and stress is important for increasing the survival and welfare of aquatic animals. The use of anesthetics is a practical approach to reduce injury and stress in aquatic animals caused by human interventions. In general, anesthetics are classified into synthetic and natural types. Crustaceans can also be anesthetized with various alcohol compounds or mixtures, although ethanol has been the most commonly used (Rotllant *et al*., 2023).

Ethanol is a drug that acts on adrenergic receptors. It is primarily a central nervous system (CNS) depressant and can cause immobility in vertebrates in response to a noxious stimulus (Wong *et al.,* 1997). Lo Bianco (1899) was the first author to propose the use of 70% alcohol to immobilize various groups of crustaceans (Ostracoda, Copepoda, Cirripedia, Cumacea, Isopoda, Amphipoda, and Schizopoda) in order to preserve them in collections to allow further studies of their morphology and anatomy. For certain groups of crustaceans (Onychopoda, Cladocera, Ostracoda, Copepoda, Isopoda, Amphipoda, and freshwater crayfish), Pennakk (1953) recommended the use of much higher concentrations (50–95%) and the addition of 5% glycerin when fixing and storing copepods.

Tricaine methane sulfate (MS 222), widely used as an anesthetic in fish, has been tested in 21 species of crustaceans with varying results. Concentrations between 0.5 and 2000 mg/L produced anesthesia in *A. franciscana*, *Diaptomus* spp., *Limnocalanus macrurus*, *Diacyclops bicuspidatus*, *Eucypris*

[Downloaded from injbir.com on 2026-02-04]

virens, *Corophium volutator*, *Echinogammarus obtusatus*, *G. pulex*, *Crangon septemspinosa*, *Hemigrapsus nudus*, *Petrolisthes cinctipes* and *Pugettia producta* with an induction time of 30 to 90 minutes. Recovery time from anesthesia ranged from 9 to 90 minutes, determined primarily by the concentration of the anesthetic bath rather than the duration of exposure. MS 222 had no anesthetic effect in *D. pulex* and other decapod species, regardless of concentration and induction time (Rotllant *et al.,* 2023). The effectiveness of MS 222 as an anesthetic does not appear to depend on taxonomy or habitat. Even within the same taxonomic group, the concentration of MS 222 required to induce anesthesia varied widely among species. For example, in the gammarid *E. obtusatus*, anesthesia was induced with 0.5 mg/L (Gamble, 1969), while in the closely related species *G. pulex*, 600 mg/L was required (Perrot-Minnot *et al.,* 2021). MS 222 is acidic and must be buffered with a similar concentration of sodium bicarbonate.

Temperature reduction has been described for a wide range of different decapod groups, including pinnipeds, caridea, clawed lobsters (Astacidea), freshwater crayfish (Astacidea), spiny lobsters (Palinuridea), hermit crabs (Anomura), and true crabs (Brachyura). Most shrimp studies have used refrigeration to improve survival during transport. Live transport technology was developed and improved in Japan by the 1960s, where wild or farmed Kuruma shrimp, *P. japonicus*, were transported live (Shigueno, 1979, 1992). This method has two stages: a pre-cooling stage, in which the animals are placed in water at 12-14°C, and then the shrimp are packed in boxes with pre-cooled sawdust (0°C or -10°C) and kept in refrigerators at 5-10°C during transport. This two-stage cooling method has also been implemented in other species of shrimp and spiny lobsters. In a research setting, ice cooling of shrimp, lobsters, freshwater crayfish, and crabs has been used in an attempt to reduce pain during surgery or tissue sampling (Rotllant *et al.,* 2023). The stages of anesthesia in crustaceans are different from those in fish and include several stages (Table 1).

Table 1: Stages of crustacean anesthesia (based on De Souza Valente, 2022).

Row	Stage/ Definition	Physiological and behavioral symptoms
1	Un anesthetized	Responsive to stimuli and awake, Normal behavior, Normal body orientation
2	Stage I-Sedation	Partial loss of equilibrium, Reduced mobility, Responsive to stimuli.
3	Stage II- Initial anesthesia	Loss of equilibrium, Lack of limb withdraw, Slow antennae withdraw, weak response to stimuli, Loss of defense behavior
4	Stage III- Surgical anesthesia	Unconsciousness, Unresponsive to stimuli, Immobility (including limb and antennae), Relaxation of abdominal flaps and chelae, Preserved scaphognathite pumping
5	Stage IV- Nervous System depression	Respiratory arrest, Cardiac arrest, Potential death

[Downloaded from injbir.com on 2026-02-04]

Despite much research conducted on the effects of various anesthetics on different species of farmed fish, there is little information on anesthesia in freshwater crayfish. This study aimed to evaluate the effect of different concentrations of ethanol, MS 222, and cold on factors such as anesthesia induction time, recovery time, and freshwater crayfish behavior in terms of anesthesia and ensuring the welfare of freshwater crayfish during manipulation and induction of stressful conditions.

Materials and methods

Freshwater crayfish (*A. leptodactylus*) with an average weight of 20±3 grams were obtained from the Aras Reservoir Lake. The *A. leptodactylus* were transferred in a uonolith and in the vicinity of ice to the culture and propagation laboratory of the National Artemia Research Center (Nekoueifard, 2016). Freshwater crayfish transferred to the laboratory were stored in 1000-liter polyethylene tanks and fed with commercial shrimp feed (Faradaneh Company) at a rate of 1% of body weight daily during storage (Mazlum *et al.,* 2011). In this study, different concentrations of ethanol (96% ethanol from Khorasan Distillation Company) and different doses of MS-222 (product of ARGENT Laboratories - USA) were used. *A. leptodactylus* weighing (20±3 g) were selected after 24 hours of starvation and transferred to plastic containers containing different levels of MS-222 (treatments 0, 50, 100, 200, 400, 800 and 1200 mg/L), ethanol (treatments 0, 5, 10, 20 and 30%) and cold (temperatures 4, 6, 8 and ambient temperature 25°C), each treatment with 3 replicates at a density of 6 individuals per replicate. The time at which the crayfish reached different stages of anesthesia, according to their appearance and behavioral characteristics, as well as the time of recovery from anesthesia, was recorded separately with a stopwatch based on Table 1, and the Mean±SD of each repetition was calculated (De Souza Valente, 2022). The total weight of crayfish was determined using a digital scale with an accuracy of 0.1 g (Seidgar *et al.,* 2022). During the experiment, the water temperature range (25±2°C) and dissolved oxygen (7±0.2 mg/L) were maintained constant by an aquarium heater and aeration.

Statistical analysis

Before performing ANOVA, the normality of the data was checked using the Kolmogorov-Smirnov test. If the data were not normal, they were transformed. One-way analysis of variance was used to analyze normal data, and after the significance of ANOVA, Duncan's test was used to compare the means of different experimental groups. The minimum significance level of the tests was $p<0.05$. The data were reported as mean ± standard deviation. SPSS version 20 software was used for statistical analysis.

Results

The time of reaching to different stages of anesthesia and recovery from anesthesia for different amounts of

[Downloaded from injbir.com on 2026-02-04]

ethanol, MS222, and cold on *A.leptodactylus* are given in Tables 2 and 3, respectively. After exposure to different levels of ethanol, *A. leptodactylus* had significant differences in the induction and recovery times from anesthesia at different concentrations of ethanol ($p<0.05$). The minimum and maximum time to reach stage III (3.97±0.35 minutes and 99.6±18.77 minutes) were observed in concentrations of 30% and 5% ethanol, respectively, with the minimum and maximum recovery time from anesthesia (1.6±4.45 minutes and 7.47±0.50 minutes), respectively. By increasing the ethanol concentration from 5% (197.6±22.91 min) to 30% (10.0±1.12 min), the time to reach stage IV (loss) was significantly reduced ($p<0.05$). Also, behavioral agitation with excessive struggling, indecision, and inappropriate response to physical stimuli (confusion) was observed in *A. leptodactylus* treated with different ethanol concentrations.

Table 2: Time of different stages of anesthesia and recovery time from anesthesia (minutes) for different amounts of ethanol on freshwater crayfish from Aras Dam.

Anesthesia stage (minutes)	Ethanol anesthetic dose (percentage per liter) at 25±2°C				
	0	5	10	20	30
I	Un anesthetized	64.3±3.06^{a}	26.0±2.0^{b}	9.17±0.76^{c}	1.90±0.36^{d}
II	Un anesthetized	74.6±3.07^{a}	33.5±3.5^{b}	13.8±1.76^{c}	2.97±0.25^{d}
III	Un anesthetized	99.6±18.77^{a}	49.3±6.03^{b}	20.33±2.52^{c}	3.97±0.35^{c}
IV	Un anesthetized	197.6±22.91^{a}	72.0±2.0^{b}	28.0±1.00^{bc}	10.0±1.12^{d}
Recovery time	Un anesthetized	1.6±4.45^{c}	1.83±0.76^{c}	5.00±2.16^{b}	7.47±0.50^{a}

The values presented in the table represent the mean ± SD. Numbers in a column with different letters are significantly different at the $p<0.05$ level.

Table 3: Time of different stages of anesthesia and recovery from anesthesia (seconds) for the effect of different temperatures on freshwater crayfish in Aras Dam

Anesthesia stage (minutes)	Storage water temperature (°C)			
	Control group (25°C)	4	6	8
I	Un anesthetized	5.20±1.25^{a}	111.7±10.4^{b}	Un anesthetized
II	Un anesthetized	10.3±1.52^{a}	226.6±15.3^{b}	Un anesthetized
III	Un anesthetized	21.67±7.63^{a}	418.3±12.6^{b}	Un anesthetized
IV	Un anesthetized	-	-	Un anesthetized
Recovery time	Un anesthetized	23.33±7.60^{a}	72.7±15.5^{b}	Un anesthetized

The values presented in the table represent the mean ± SD. Numbers in a column with different letters are significantly different at the $p<0.05$ level.

Exposure to temperatures of 4 and 6°C resulted in a significant difference in the time to induction of anesthesia and successful recovery from anesthesia. The time to reach stage III anesthesia increased from 21.7±7.63 seconds at 4°C to 418.3±12.6 seconds at 6°C. Also, the recovery time from anesthesia increased from 23.33±7.60 seconds at 4°C to 72.7±15.5 seconds at 6°C. The temperature of 8°C and 25°C had no effect on the anesthesia of freshwater crayfish from Aras Dam, so that even storage for 24 hours could not bring the tested crayfish to stage I anesthesia.

[Downloaded from injbir.com on 2026-02-04]

None of the crayfish reached stage IV anesthesia at the temperatures studied.

Exposure to different concentrations of MS-222 showed that concentrations of 50, 100, 200, and 400 mg/L of MS-222 had no effect on anesthesia of the freshwater crayfish studied. The concentration of 800 mg/L was able to achieve stage I anesthesia only after 269.00 ± 24.00 minutes, while only concentrations above 1200 mg/L resulted in stage III anesthesia after prolonged exposure to the anesthetic (1440.0±80.8 minutes). None of the crayfish reached stage IV anesthesia at different concentrations of MS222. The recovery time from anesthesia in this case was recorded as 5.4±0.96 minutes.

Discussion and conclusion

The above study provides indicators of different levels of anesthesia and a comprehensive assessment of the time of anesthesia induction and recovery from anesthesia in freshwater crayfish from Aras Dam using common anesthetics in crustaceans, including ethanol, MS222, and cold, and determining their appropriate doses. Decapod crustaceans (crabs, hermit crabs, lobsters, crayfish, shrimp) are intelligent creatures that not only respond to noxious stimuli, but are also capable of feeling pain, discomfort, and distress. The choice of anesthetic agents depends on the species of animal, the animal's weight, the availability of equipment, the skill of the veterinarian, and the requirements of the procedure, which determine the duration and depth of anesthesia required. Similarly, the dose and time of exposure to anesthesia, environmental factors, and the animal's body weight, sex, and physiological status directly affect the effectiveness of anesthesia. For example, oviparous females or wild animals may have a longer induction time and a faster recovery time than non-oviparous females or animals previously kept in captivity (Cowing *et al.,* 2015). Similarly, induction and recovery times increase with increasing animal body weight and air exposure time (Li *et al*., 2018; Ghanawi *et al.,* 2019; Jiang *et al.*, 2020). The lower the animal's body weight, the greater the oxygen consumption and, as a result, the higher the concentration of anesthetic agent per entering the body, resulting in a shorter induction time (Jiang *et al.,* 2020). Also, induction time decreases with increasing water temperature and high anesthetic concentration (Li *et al.,* 2018).

Anesthetics are important in inducing relaxation in aquatic animals and reducing stress during transportation and handling. However, no study has been conducted to date on the effect of anesthetics on the freshwater crayfish: *A. leptodactylus* in the Aras Dam. Clove oil, MS-222, ethanol, and magnesium chloride have been used in crustaceans such as blue crabs, crayfish, gammarids, Pacific white shrimp, and European lobsters (Stanley *et al.,* 2020; Perrot-Minnot *et al.,* 2021; De Souza Valente, 2022; Rotllant *et al.,* 2023). However, survival rates in crabs exposed to MS-222 and magnesium chloride were reported to be lower than those exposed to clove oil and ethanol. Therefore, MS222 and magnesium chloride are not

[Downloaded from injbir.com on 2026-02-04]

considered effective anesthetics in crabs (Zhu *et al.,* 2023), which is consistent with the results of the present study regarding the ineffectiveness and effectiveness of MS-222 on freshwater crayfish anesthesia. Previous studies have shown that *Crangon septemspinosa* is anesthetized by 0.5 g/L of MS-222. In comparison, immersion in MS-222 in freshwater crayfish was found to be ineffective at low concentrations (100 mg/L) and had only a mild effect at high concentrations (1000 mg/L). The latter concentration is lethal in fish. This is probably due to differences in neurotransmitters present in crustaceans (Ross and Ross, 2008, Mirzargar and Seidgar, 2023). The ineffectiveness of MS-222 as an injectable anesthetic, volatile, or applied by immersion has also been reported in a number of previous studies (Oswald, 1977; Obradovič, 1986; O'Brien, 2008; Mosley and Lewbart, 2014). In this regard, the results of the present study showed that MS-222 was able to induce stage III anesthesia in freshwater crayfish only at levels above 1200 mg/L after long-term exposure. Some methods and chemicals are known to be inadequately analgesic, or their analgesic and anesthetic effects in decapod crustaceans are questionable. For example, hypothermia (reducing body temperature) does not provide pain relief, but only induces immobility and lethargy, meaning that even though the animal is immobile, sensation and response to painful stimuli are still present (Pellet *et al.,* 2020; Gressler *et al*., 2021). Hypothermia should never be used or tolerated for any painful or invasive procedure (Cooper, 2004). Cooling may also result in self-amputation of appendages (Oswald, 1977; O'Brien, 2008). Given the fact that low temperatures do not activate pain receptors in some crustaceans, ice immersion appears to be a highly effective, easy, non-chemical method of humane immobilization. However, this method is not recognized by law in some countries, for example, Switzerland or Italy. In fact, there have been suspicions that cold alone may paralyze animals without anesthesia. Electrical anesthesia induces epileptic seizures in lobsters and crayfish. Relatively low temperatures of 5 to 10°C, a humid environment, and avoiding disturbance of crustaceans during transportation enhance their welfare (Ross and Ross, 2008; Mirzargar and Seidgar, 2023). According to the results of the present study, in all anesthetic drugs used, including MS-222, ethanol, the induction and recovery time from anesthesia was in minutes, while the use of cold (temperatures of 4 and 6 degrees Celsius) resulted in induction and recovery time from anesthesia in a much shorter time than other anesthetic drugs. Due to the endemicity of the freshwater crayfish of Aras Dam, information about the medication, method of administration, induction of anesthesia, and recovery time from anesthesia was not available for this species. Therefore, choosing scientifically informed methods of sedation/anesthesia to reduce pain or stress during any manipulation, including transportation, research,

[Downloaded from injbir.com on 2026-02-04]

surgery, will improve their well-being and, with humane treatment, can reduce stress and pain. Some methods and chemicals were unsuitable for use as anesthetics, and most drugs required much higher doses to induce anesthesia in freshwater crayfish than in fish. Given the perception of pain in freshwater crayfish, it is recommended that these animals be treated humanely; while observing the principles of care and stress and pain management to maintain their welfare and wellbeing Also, methods or chemicals that cause adverse reactions should be avoided. Further research is also needed to evaluate the impact of other anesthetics, age, growth stage, sex, temperature, pH, and salinity on the effectiveness of a selected method or drug. If the purpose of anesthesia is to transport freshwater crayfish from Aras Dam (*A. leptodactylus*) to the market for human consumption, transport at low temperature (4-6°C in the vicinity of ice) is recommended. On the other hand, the use of ethanol is not recommended due to stress induction and mortality, the need for high doses, high price, long exposure time required to induce anesthesia, and the use of MS-222 is not recommended due to high price and low performance in crustaceans compared to cold water exposure.

Acknowledgement

We hereby express our gratitude for the support of the National Fisheries Science Research Institute.

References

Cooper, J.E., 2004. Invertebrate care. Veterinary Clinics: Exotic Animal Practice, 7(**2**), 473-486.

Cowing, D., Powell, A. and Johnson, M., 2015. Evaluation of different concentration doses of eugenol on the behavior of *Nephrops norvegicus*. *Aquaculture*, 442, 78–85. https://doi.org/10.1016/j.aquaculture.2015.02.039.

De Souza Valente, C., 2022. Anaesthesia of decapod crustaceans. *Veterinary and Animal Science*, 16(**100252**), 1-15. https://doi.org/10.1016/j.vas.2022.100252.

Gamble, J., 1969. An anaesthetic for *Corophium volutator* (pallas) and *Marinogammarus obtusatus* (dahl), *Crustacea, Amphipoda. Experientia*, 25, 539–540.

Ghanawi, J., Saoud, G., Zakher, C., Monzer, S. and Saoud, I.P., 2019. Clove oil as an anesthetic for Australian red claw crayfish *Cherax quadricarinatus*. *Aquaculture Research*, 50, 3628–3632. https://doi.org/10.1111/are.14319.

Ghorbanzadeh, R.A. and Sedghpour, S., 2024. Statistical Yearbook of the Iranian Fisheries Organization 1402-1398. Iranian Fisheries Organization, Deputy for Planning and Resource Management/Planning and Budget Office/Planning and Statistics Group. 64 P.

Gressler, L.T., Heinzmann, B.M. and Baldisserotto, B., 2021. Analgesia, anesthesia, and euthanasia of aquatic animals. In F.S.B. Kibenge, B. Baldisserotto, & R.S.M. Chong (Eds.), *Aquaculture Pharmacology*, pp. 297–

[Downloaded from injbir.com on 2026-02-04]

346. Academic Press. https://doi.org/10.1016/B978-0-12-821339-1.00007-6.

Jiang, S., Zhou, F., Yang, W., Wu, Z., Le, Y., Yang, Q., Yu, Y. and Jiang, S., 2020. In aesthetic effect of eugenol at different concentrations and temperatures on black tiger shrimp (*Penaeus monodon*). *Aquaculture Research*, 51, 3268–3273. https://doi.org/10.1111/are.14662.

Li, Y., She, Q., Han, Z., Sun, N., Liu, X. and Li, X., 2018. Anaesthetic effects of eugenol on Grass Shrimp (*Palaemonetes sinensis*) of Different Sizes at Different Concentrations and Temperatures. *Scientific Reports*, 8(**1**), 11007, https://doi.org/10.1038/s41598-018-28975-w.

Lo Bianco, S., 1899. The methods employed at the Naples Zoological Station for the preservation of marine animals. *Bulletin of the United States National Museum,* 39, 1–42.

Mazlum, Y., Güner, Ö. and Şirin, S., 2011. Effects of feeding interval on growth, survival and body composition of narrow-clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823 juveniles. *Turkish Journal of Fisheries and Aquatic Sciences,* 11, 283-289. DOI:10.4194/trjfas.2011.0213.

Mirzargar, S.S. and Seidgar, M., 2023. Anaesthetic and Sedative Techniques in Aquatic Animals. Translation of the second edition. University of Tehran Press. 228 P.

Mosley, C.I. and Lewbart, G.A., 2014. Invertebrates. In G. West, D. Heard, & N. Caulkett (Eds.), zoo animal and wildlife immobilization and anesthesia (pp. 191–208). John Wiley & Sons. Online ISBN:9781118792919.

Nekoueifard, A., 2016. Investigation of the effects of transportation and storage under frozen conditions on the meat quality of freshwater crayfish. Project approved by the National Fisheries Science Research Institute, registration number 48583, 40 P.

Nekoueifard, A., Valinasab, T., Seyed Mortazaei, S.R., Fasih, M., Mokhayer, Z., Talebzadeh, A., Valipour, A.R., Imani, A., Mohebbi, F., Abdy, K., Mohsen pour Azari, A., Manaffar, R., Seidgar, M., Shiri, S., Mostafazadeh, B., Abbas pour Anbi, A., Alizadeh Osalou, Zh. and Ganji Golmankhaneh, S., 2019. Review of the latest scientific and practical findings on the reproduction, culture, assessment and restoration of freshwater crayfish stocks and diseases. Final report approved by the National Fisheries Science Research Institute. Registration number 56342. 94 P.

O'Brien, M., 2008. Invertebrate anaesthesia. In L. Longley (Ed.), Anaesthesia of exotic pets (pp. 279–295). Elsevier. https://doi.org/10.1016/B978-0-7020-2888-5.50001-0.

Obradovič, J., 1986. Effects of anaesthetics (halothane and MS-222) on crayfish, *Astacus astacus*. *Aquaculture*, 52(**3**), 213–217. https://doi.org/10.1016/0044-8486(86)90146-8.

Oswald, R.L., 1977. Immobilization of decapod crustacea for experimental procedures. *Journal of the Marine Biological Association of the United*

[Downloaded from injbir.com on 2026-02-04]

Kingdom, 57(**3**), 715–721. https://doi.org/10.1017/S0025315400025121.

Pennak, R.W., 1953. Freshwater invertebrates of the United States; The Ronald Press Company: New York, NY, USA. 112 P.

Perrot-Minnot, M.J., Balourdet, A. and Musset, O., 2021. Optimization of anesthetic procedure in crustaceans: Evidence for sedative and analgesic-like effect of MS-222 using a semi-automated device for exposure to noxious stimulus. *Aquatic Toxicology,* 240, 105981. https://doi.org/10.1016/j.aquatox.2021.105981.

Ross, L. and Ross, B., 2008. Anaesthetic and sedative techniques for aquatic animals, 3rd Edition. Wiley-Blackwell. 240 P.

Roth, B. and Skra, T., 2021. Pre mortem capturing stress of Atlantic herring (*Clupea harengus*) in purse seine and subsequent effect on welfare and flesh quality. *Fisheries Research*, 244, 106124.

Seidgar, M., Shiri, S., Nekouiefard, A., Valipour, A.R. and Mohebbi, F., 2022. The rirst report of an unusual freshwater crayfish (*Astacus leptodactylus*) in Aras Dam of Iran: A case study. *American Journal of Zoology,* 5, 2, 2022, pp. 20-23.

Shigueno, K., 1979. Problems in prawn culture; Aquaculture series 19; Balkema Press: Rotterdam, The Netherlands, Volume 19, pp. 1–103.

Shigueno, K., 1992. Shrimp culture industry in Japan. In marine shrimp culture: Principles and practices; Fast, A.W., Lester, J., Eds.; The Elsevier: Amsterdam, The Netherlands, Volume 30, pp. 641–652.

Stanley, C.E., Adams, R., Nadolski, J., Amrit, E., Barrett, M., Bohnett, C. and Cooper, R.L., 2020. The effects of tricaine mesylate on arthropods: Crayfish, crab and drosophila. *Invertebrate Neurosciences*, 20, 10.

Wong, S.M.E., Fong, E., Tauck, D.L. and Kendig, J.J., 1997. Ethanol as a general anesthetic: Actions in spinal cord. *European Journal of Pharmacology*, 329, 121–127. https://doi.org/10.1016/S0014-2999(97)89174-1.

Zhu, L., Qi, S., Shi, C., Chen, S., Ye, Y.,Wang, C., Mu, C., Li, R., Wu, Q.,Wang, X. and Zhou, Y., 2023. Optimizing anesthetic practices for mud crab: A comparative study of clove oil, MS-222, ethanol, and magnesium chloride. *Antioxidants*, 12, 2124. https://doi.org/10.3390/antiox12122124 .

[Downloaded from injbir.com on 2026-02-04]