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Comparative Efficacy of Anesthetics for the Freshwater Prawn *Macrobrachium rosenbergii*

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Abstract

The freshwater prawn *Macrobrachium rosenbergii* is a commercially important culture species in the South Central United States. Two major constraints in the commercial culture of the freshwater prawn in the U.S. are poor survival during live transportation of seed-stock to growout ponds, and live transportation of pond harvested prawn to distant live markets due to the territorial and cannibalistic nature of prawn. The use of anesthetics could possibly improve transport survival; however, to date anesthetic agents have not been evaluated for use with prawn. Two trials were conducted with juvenile freshwater prawn to compare the efficacy of anesthetics commonly used on fish. The first trial was designed to identify the most promising candidates. In Study 1, tricaine methanesulfonate (MS-222), 2-phenoxyethanol, quinaldine sulfate (quinaldine), clove oil, and Aqui-S™ were evaluated at 25 and 100 mg/L for 1 h in three replicate 10-L glass containers, containing five juvenile prawn each. Relative sedation level was determined every 3 min for 1 h, then recovery time and survival were measured. In Study 1, MS-222 and 2-phenoxyethanol were determined to be ineffective on prawn at all rates tested. Based on their performance in Study 1, quinaldine, clove oil, and Aqui-S™ were evaluated at 100, 200, and 300 mg/L in Study 2. Observations were determined as in Study 1. Clove oil and Aqui-S™ induced anesthesia faster and at lower concentrations than quinaldine. At the highest treatment rate (300 mg/L) prawn suffered 60% mortality in the Aqui-S™ treatment, 13% mortality in the quinaldine treatment, and 0% mortality in the clove oil treatment and control following a 1-h exposure to these concentrations. Based on these data, Aqui-S™ and clove oil applied at 100 mg/L may be suitable anesthetic treatments for prawn. Additional research is needed to determine optimal time and dose relationships to minimize stress during holding, handling, and transportation of prawn.

The freshwater prawn *Macrobrachium rosenbergii* is a commercially important culture species in the South Central United States with an estimated 400 ha of pond production (Tidwell and D'Abramo 2003). Two major constraints in the commercial culture of the freshwater prawn in the United States are poor survival during live transportation of juvenile prawn from the nursery to grow-out ponds and post-harvest transportation of prawn to distant live markets (Coyle et al. 2001). Prawn are territorial and cannibalistic, especially under crowded conditions; mortality during transport is thought to be largely due to negative interactions within prawn populations (Smith and Wannamaker 1983; Alias and Siraj 1988). This behavior limits its carrying capacity under transport conditions to 10–20 g/L for prawn (Smith and Wannamaker 1983; Alias and Siraj 1988; and Coyle et al. 2001) compared to 40–600 g/L for various warmwater fish species (Jensen 1990).

Juvenile freshwater prawn (0.3–0.6 g; D'Abramo et al. 1995) are transported from nursery facilities to production ponds for grow-out. Stress during this transport is thought to be the cause of undetected mortalities after prawn are stocked into ponds (Coyle et al. 2001). This can result in reduced pond stocking density, reduced pond production, poor feed conversion efficiency, and deteriorated water quality due to accumulation of uneaten feed. Survival in commercial ponds in temperate regions of the U.S. is typically 50–60% in a 4–5 mo growing season (Coyle and Tidwell 2003). However, survival in research ponds where prawn are nursed on-site and not transported to distant grow-out facilities is typically 85–90% (Tidwell et al. 1996, 1997, 1998, 1999, 2000). Critical to

the success of prawn farming in temperate climates is improved survival through pond grow-out. Reducing post-stress mortality following the transport of prawn juveniles from nursery facilities to grow-out ponds could greatly improve apparent grow-out survival.

The potential exists for sales of large volumes of live freshwater prawn in Asian communities located in urban areas of the U.S. and Canada (Tidwell and D'Abramo 2000). In these regions, farm-gate prices for live freshwater prawn achieve US $13.2–22.0/kg (Tidwell and D'Abramo 2000) compared to the average price of approximately US $8.0/kg for imported processed shrimp (Harvey 2003). While the demand for live product remains strong, initial efforts to supply these markets have been hampered by poor prawn survival during transport, and during wholesale and retail product distribution and display. Poor survival has been attributed to deteriorated water quality and predation in transport and display containers (Coyle and Tidwell 2003). If methodologies can be developed to reduce stress during transport, a large market exists for live product in these urban areas.

The use of anesthetics has been reported to increase transport densities and duration in finfish (Summerfelt and Smith 1990). A number of anesthetics have been evaluated experimentally. Some of these anesthetics (i.e., quinaldine, 2-phenoxyethanol) are illegal for use on food-fish in the United States; however, they are used on non-food fish and in research. Currently, the only anesthetics approved by the U.S. Food and Drug Administration (FDA) for use on aquaculture food products in the United States are tricane methanesulfonate (MS-222) and carbon dioxide (CO_2) (Schnick et al. 1986). Tricane has a 21-d withdrawal period before fish can be consumed, and carbon dioxide may not produce sufficient anesthesia and has a relatively narrow margin for safety (Summerfelt and Smith 1990). Alternative anesthetics need to be developed for aquaculture that are cost effective, safe, efficacious, and that could be registered by the FDA for rested harvest and transport of live product for immediate sale as food.

Recently, much interest has been devoted to the study of clove oil as an anesthetic for fish. A solution of 10% clove oil mixed with 90% ethanol is reported to be an effective anesthetic at application rates between 25–150 mg/L for most fish species (Soto and Burhanuddin 1995; Anderson et al. 1997; Taylor and Roberts 1999). Clove oil is 85–90% eugenol, with the remaining 5–15% made up of isoeugenol and methyleugenol. Eugenol is used as an anesthetic in human medicine and dentistry and is cleared for use as a topical anesthetic for use on humans by the FDA (Nagababu and Lakshmaiah 1992; Soto and Burhanuddin 1995). Although not approved as a new animal drug for general use as a fish anesthetic in the U.S., clove oil has been affirmed as Generally Recognized as Safe (GRAS) and can be added directly to human food (FDA 2002). Aqui-S™ is a relatively new product approved for use in aquatic species in New Zealand and Australia with 0-withdrawal time and is currently undergoing the New Animal Drug Act (NADA) approval process for use in the United States. The active ingredient in Aqui-S™ is isoeugenol, which is a component of clove oil. These anesthetics appear to have the greatest potential for approval for use with 0-withdrawal time and should be evaluated for their effectiveness for use as anesthetics in freshwater prawn.

To date, a limited amount of research has been conducted on the use of anesthetics in crustaceans. Brown et al. (1996) reported that MS-222 concentrations as high as 1,000 mg/L had no effect on crayfish *Oronectes virilis*. Ozeki (1975) reported eugenol, the active ingredient of clove oil, to be an effective anesthetic for crayfish *Procambarus clarkii* at doses of 200–1000 ppm. Morgan et al. (2001) evaluated clove oil as an anesthetic for use on three Pacific coast crab species (dungeness crab *Cancer magister;* hairy shore crab *Hemigrapsus oregonensis;* and northern kelp crab *Pugettia producta*) and indicated great differences in the application rates required to induce anesthesia for the different species. No references were found related to the use of anesthetics with the freshwater prawn *Macrobrachium rosenbergii*.

The objectives of the present research were

to evaluate the potential of five commonly used fish anesthetics for use with the freshwater prawn (Study 1), and then determine appropriate treatment concentrations for the most promising candidates (Study 2).

Materials and Methods

Study 1

Preliminary screening trials were conducted to identify candidate compounds and appropriate ranges of dose-concentrations for use with the freshwater prawn. Preliminary trials (Study 1) evaluated quinaldine sulfate (quinaldine) (Surelife Laroratories Corp., Seguin, Texas, USA), clove oil (Sigma Chemical Co., St. Louis, Missouri, USA), Aqui-S™ (AQUI-S New Zealand LTD, Lower Hutt, New Zealand), tricaine methanesulfonate (MS-222) (Argent Chemical Laboratories, Inc., Redmond, Washington, USA) and 2-phenoxyethanol (Mallinckrodt Baker, Inc., Phillipsburg, New Jersey, USA) at 25 and 100 mg/L.

Study 2

Based on their performance in the first trial, quinaldine, clove oil, and Aqui-S™ were evaluated at 100, 200, and 300 mg/L in Study 2.

Experimental System

In both studies, there were three replicate aquaria per anesthetic treatment and three control aquaria (no anesthetic). In Study 1, the system consisted of 33, 10-L glass aquaria (including three control tanks) each filled with 6-L of municipal water that had been aerated for 24 h in a 400-L plastic tank to remove chlorine. In Study 2, the system was the same except 30 aquaria were used including the three control tanks. Each aquarium contained an air stone supplied by a regenerative blower.

Application rates were based on the active ingredient for each compound. The compounds were applied to aerated containers 5 min prior to stocking prawn to allow anesthetic agents to mix thoroughly. Five juvenile prawn (2.0 g ± 0.1 for Study 1; 2.1 g ± 0.1 for Study 2) were then randomly stocked into each aquarium.

The relative sedation of individual prawn was ranked based on observations made at 3-min intervals. The rankings ranged from 0–2. Every 3 min all prawn in each aquarium were given tactile stimulation, by prodding with a plastic straw to stimulate a reaction, and observations were recorded for each prawn. A ranking of 0 was given to prawn that demonstrated no response to the anesthetic and a normal escape response to touch stimuli. A ranking of 1 was given to prawn that demonstrated a partial loss of equilibrium, but was still reactive to touch stimuli. A ranking of 2 was given to prawn demonstrating a complete loss of equilibrium and not reactive to stimuli.

The total duration of this study was 1 h. If all the prawn in any aquaria were ranked at a sedation level of 2, they were moved into recovery tanks containing aerated freshwater for 1 h. During recovery from anesthesia, the most apparent stage occurred when prawn first regained control of equilibrium and attained an upright position on the bottom of the tank. This was an unambiguous response and was adopted as the level of recovery for comparison among treatments.

Water quality analysis was performed using a YSI 85 oxygen meter (YSI, Yellow Springs, OH, USA) and Odyssey DR/2500 spectrophotometer (HACH Company, Loveland, Colorado, USA). Water quality was taken from a common water source and run prior to the completion of each study. Water quality conditions for Study 1 were: temperature, 24.0 C; total dissolved oxygen, 8.3 mg/L; total ammonia-nitrogen, 0.34 mg/L; total nitrite-nitrogen, 0.002 mg/L; and pH, 7.3. Water quality conditions for Study 2 were: temperature, 23.7 C; total dissolved oxygen, 8.5 mg/L; total ammonia-nitrogen, 0.29 mg/L; total nitrite-nitrogen, 0.002 mg/L; and pH, 7.5.

Statistical Analysis

Study 1 evaluated the effects of five chemicals (quinaldine, clove oil, Aqui-S™, MS-222, and 2-phenoxyethanol) each applied at 25 and 100 mg/L, respectively for 15 to 60 min on juvenile freshwater prawn. This study was conducted to identify which chemicals (and appropriate concentrations) were effective in anesthetizing prawns.

Experimental data from Study 2 were organized into the following dependent and independent variables: 1) proportion of prawn in Stages 0, 1, and 2 per aquarium (dependent), 2) dummy variables representing chemical treatments (quinaldine, Aqui-S™, and clove oil), 3) chemical concentration (100 mg/L, 200 mg/L, or 300 mg/L); and 4) time elapsed in minutes. The data from Study 2 were used in an Ordered Probit regression to explain how the independent variables affected the probability of prawns being in different stages of anesthesia. The regression model was developed in the following manner: if y_i represented the anesthesia state of a prawn (y_i = 0: no response, 1: partial loss of equilibrium, 2: complete loss of equilibrium), it can be interpreted as the observed effects of an underlying, continuous, latent variable (y^*_i), which symbolizes the unobserved level of anesthesia of prawn. If y^*_i were a linear function of the applied anesthetic, its concentration, and time, then $y^*_i = \beta_0 * DVQ + \beta_1 * DVA + \beta_2 * DVC + \beta_3 * Conc + \beta_4 * Time + error = \beta'X + error$, where DVQ – DVC are dummy variables for quinaldine, Aqui-S™, and clove oil, respectively. Using the theory of ordinal discrete dependent variable regressions y is related to y* in the following manner: $y_i = 0$ if $y^*_i \leq 0$, $y_i = 1$ if $0 < y^*_i \leq \mu$, and $y_i = 2$ if $y^*_i > \mu$, where μ is a threshold parameter (Greene 1990). Although individual data for the dependent variable were unavailable, proportional data (as described above) were sufficient to develop an Ordered Probit regression model in LIMDEP 7 (Econometric Software Inc. 1998), which explained the variation in the proportion of prawn in different stages of anesthesia with respect to the independent variables described above.

Using the estimated β's from the above model, the probabilities of prawn being in the three stages of anesthesia were $P(y = 0) = 1 - \Phi(\beta'X)$, $P(y = 1) = \Phi(\mu - \beta'X)$, $P(y = 2) = 1 - \Phi(\mu - \beta'X)$, where Φ is the standard normal cumulative distribution function. Using these probability formulae, one could derive the marginal effects of each regressor (i.e., $\partial P(y = i)/\partial x$, for I = 0, 1, and 2), which would clarify the effects of each independent variable on the likelihood of anesthesia of prawn.

Survival and recovery data were analyzed by analysis of variance (ANOVA) using Statistix version 7.0 (Analytical Software, Tallahassee, Florida, USA) to determine treatment effects on recovery time and survival. If ANOVA indicated significant treatment effects, the Least Significant Difference test (LSD) was used to determine differences among means ($P \leq 0.05$). All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Data are presented in the untransformed form to facilitate interpretation.

Results and Discussion

Study 1

In Study 1, MS-222 and 2-phenoxyethanol were found to be ineffective for use as anesthetic agents with freshwater prawn at the treatment rates tested (Table 1). This is in agreement with Brown et al. (1996) who found that MS-222 concentrations as high as 1000 mg/L had no effect on crayfish *Oronectes virilis*. Treatment rates of 25–100 mg/L with clove oil, Aqui-S™ and quinaldine proved to be only slightly effective at sedating prawn (Table 1); therefore, increased concentrations of these compounds (100, 200, and 300 mg/L) were evaluated in Study 2.

Study 2

In Study 2, quinaldine, clove oil, and Aqui-S™ were all effective in immobilizing prawn. Induction times and levels of sedation were dose dependant and varied considerably between the three treatments (Table 2). The time required for induction of anesthesia was reduced as the concentration of anesthetics increased from 100 to 300 mg/L. Table 3 shows survival and recovery times for the different anesthetics and dose concentrations tested. Clove oil resulted in longer recovery times than quinaldine or Aqui-S™. Aqui-S™ resulted in lower survival, following the recovery period, than quinaldine or clove oil.

Results from the Ordered Probit regression showed that all three anesthetics had significantly different effects on the anesthetic state of prawn. Table 4 shows that Aqui-S™ and

TABLE 1. *The percent relative rank of freshwater prawn* Macrobrachium rosenbergii *exposed to different anesthetics applied at either 25 or 100 mg/L where: P0 = prawn that demonstrated no response to the anesthetic and a normal escape response to touch stimuli, P1 = prawn that demonstrated a partial loss of equilibrium, but were still reactive to touch stimuli, and P2 = prawn demonstrating a complete loss of equilibrium and not reactive to stimuli. These are observations made at 15-min intervals.*

Anesthetic	mg/L	15 min P0/P1/P2	30 min P0/P1/P2	45 min P0/P1/P2	60 min P0/P1/P2
Control	0	100/0/0	100/0/0	100/0/0	100/0/0
Clove Oil	25	100/0/0	100/0/0	100/0/0	93.3/6.7/0
Clove Oil	100	100/0/0	100/0/0	100/0/0	86.7/13.3/0
Aqui-S™	25	100/0/0	100/0/0	100/0/0	100/0/0
Aqui-S™	100	66.6/33.3/0	0/86.7/13.3	0/86.7/13.3	0/86.7/13.3
Quinaldine	25	100/0/0	60/13.3/26.7	26.7/13.3/60	0/20/80
Quinaldine	100	100/0/0	0/100/0	0/100/0	0/100/0
MS-222	25	100/0/0	100/0/0	100/0/0	100/0/0
MS-222	100	100/0/0	100/0/0	100/0/0	100/0/0
2-Phenoxyethanol	25	100/0/0	100/0/0	100/0/0	100/0/0
2-Phenoxyethanol	100	100/0/0	100/0/0	100/0/0	100/0/0

TABLE 2. *The percent relative rank of freshwater prawn* Macrobrachium rosenbergii *exposed to different anesthetics applied at either 100, 200, or 300 mg/L where: P0 = prawn that demonstrated no response to the anesthetic and a normal escape response to touch stimuli, P1 = prawn that demonstrated a partial loss of equilibrium, but were still reactive to touch stimuli, and P2 = prawn demonstrating a complete loss of equilibrium and not reactive to stimuli. These are observations made at 15-min intervals.*

Anesthetic	mg/L	15 min P0/P1/P2	30 min P0/P1/P2	45 min P0/P1/P2	60 min P0/P1/P2
	Control	0	100/0/0	100/0/0	100/0/0
100/0/0					
Clove Oil	100	0/100/0	0/100/0	0/100/0	0/86.7/13.3
Clove Oil	200	0/100/0	0/46.7/53.3	0/13.3/86.7	0/0/100
Clove Oil	300	0/53.3/46.7	0/33.3/66.7	0/0/100	0/0/100
Aqui-S™	100	66.7/33.3/0	13.3/86.7/0	0/60/40	0/53.3/46.7
Aqui-S™	200	0/73.3/26.7	0/20/80	26.7/6.7/66.7	0/13.3/86.7
Aqui-S™	300	0/80/20	0/33.3/66.7	0/13.3/86.7	0/0/100
Quinaldine	100	100/0/0	60/33.3/6.7	40/53.3/6.7	26.7/60/13.3
Quinaldine	200	86.7/13.3/0	40/60/0	20/73.3/26.7	40/80/20
Quinaldine	300	33.3/66.7/0	0/100/0	6.7/73.3/26.7	40/80/20

clove oil had similar effects on the dependent variable (i.e., the marginal effects for both these chemicals significantly increased the likelihood of prawn reaching Stage 2 of anesthesia). However, in contrast, quinaldine had a substantially weaker impact on the anesthetic state of prawn. Marginal effects, computed at the mean values of other independent variables, show that quinaldine was only effective in causing prawn to reach Stage 1 of anesthesia. The marginal effects of other independent variables, such as chemical concentration and time, were predictable. The likelihood of prawn arriving at Stage 2 of anesthesia improved with higher chemical concentrations and additional time.

The above results are further illustrated in Fig. 1. The three graphs in the figure show the progression of the latent variable y*, representing anesthesia of prawn, with respect to chemicals, and time. Here, Aqui-S™ and clove oil were kept at a low concentration (100 mg/L), and quinaldine was kept at a high concentration (300 mg/L), for purposes of comparison. Clearly, Aqui-S™ and clove oil were capable of

TABLE 3. *Time required to recover from anesthesia (Recovery Time) and survival of freshwater prawn* Macrobrachium rosenbergii *exposed to clove oil, Aqui-S™, and quinaldine when applied at 100, 200, and 300 mg/L. Values are means ± SD of three replicates. Means within a column followed by different letters are significantly different (P < 0.05) by ANOVA.*

Anesthetic	Rate (mg/L)	Recovery Time (min)	Survival (%)
Clove Oil	100	18.3 ± 2.9 de	100.0 ± 0.0 a
Clove Oil	200	85.0 ± 13.2 a	100.0 ± 0.0 a
Clove Oil	300	70.0 ± 25.0 ab	100.0 ± 0.0 a
Aqui-S™	100	28.3 ± 12.6 cde	93.3 ± 11.5 ab
Aqui-S™	200	36.7 ± 5.8 cd	73.3 ± 11.5 b
Aqui-S™	300	36.7 ± 20.2 cd	40.0 ± 32.7 c
Quinaldine	100	11.7 ± 7.6 e	100.0 ± 0.0 a
Quinaldine	200	50.0 ± 0.0 bc	100.0 ± 0.0 a
Quinaldine	300	18.3 ± 11.5 de	86.7 ± 11.5 ab

TABLE 4. *Results of ordered probit regression of the proportion of prawn in the three stages of anesthesia. Data came from Study 2, with N = 141. Likelihood ratio index = 0.47.*

Regressor`	Coefficient estimate	Expected value / SE	Marginal effects
DVA	-3.80	-1.78[a]	P(y=0): 0.64→0; P(y=1): 0.37→0.23; P(y=2): 0→0.77
DVC	-2.66	-1.02	P(y=0): 0.54→0; P(y=1): 0.46→0.01; P(y=2): 0→0.99
DVQ	-6.90	-2.71[b]	P(y=0): 0.34→0.21; P(y=1): 0.66→0.79; P(y=2): 0→0
DVA*Time	0.33	3.18[b]	_[c]
DVC*Time	0.30	2.73[b]	_[c]
DVQ*Time	0.34	3.03[b]	_[c]
Concentration	$0.51 * 10^{-2}$	2.01[b]	P(y=0): $-3.61 * 10^{-4}$; P(y=1): 1.59×10^{-4}; P(y=2): $2.01 * 10^{-4}$
Time^2	$-0.35 * 10^{-2}$	-2.57[b]	_[d]
μ	4.00	4.31[b]	

[a] *P-value < 0.10.*

[b] *P-value < 0.05.*

[c] *Marginal effects for the "dummy variable*Time" regressors were incorporated into the marginal effects for the corresponding dummy variable regressors, evaluated at the mean time for the data sample.*

[d] *Marginal effects (ME) for time are dependent on the values of several regressors: ME(P(y=0)) = -ϕ(-β□X)*[0.33*DVA +0.30*DVC+0.34*DVQ-0.70*10^{-2}*time]; ME(P(y=1)) = -[ϕ(μ - β□X) - ϕ(-β□X)]*[0.33*DVA+0.30*DVC+0.34*DVQ-0.70*10^{-2}*time]; ME(P(y=2)) = ϕ(μ - β□X) *[0.33*DVA+0.30*DVC+0.34*DVQ-0.70*10^{-2}*time], where ϕ is the standard normal probability distribution function.*

making prawn reach Stage 2 within the first 30 minutes. The data also showed that, on average, quinaldine was not very effective, even at high concentrations, in causing prawn to reach Stage 2 of anesthesia.

Clove oil required less time to induction and required a longer recovery than Aqui-S™ and quinaldine, which could prove to be advantageous for transport. This is in agreement with Munday and Wilson (1997) who found recovery time after anesthesia with clove oil was two to three times longer in the coral reef fish *Pomacentrus amboinensis* than recovery from other chemicals. Additionally, prawn treated with clove oil had 100% survival at the end of the recovery time for all treatment rates. Ozeki (1975) found that crayfish *Procambarus clarkii* exposed to eugenol, the active ingredient of clove oil, at doses of 200–1000 mg/L were able to recover from the anesthetic effects with no

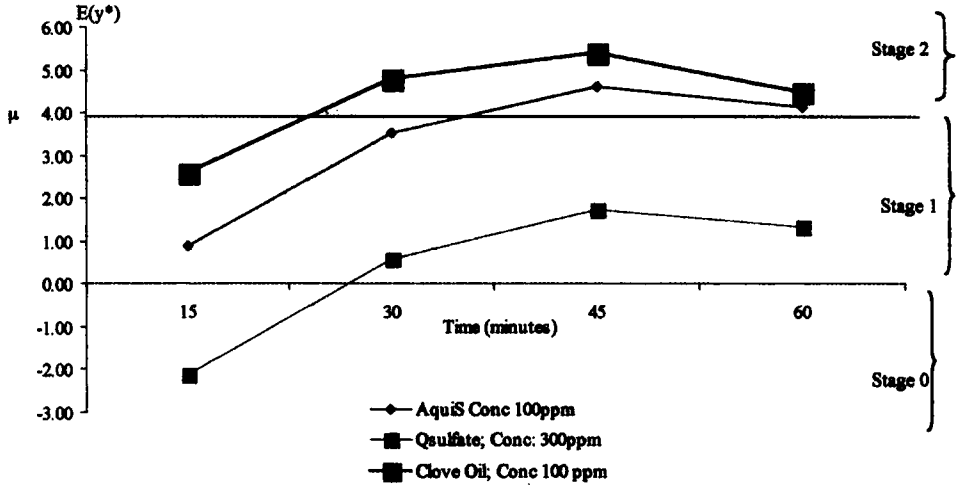

FIGURE 1. *Predicted stage of anesthesia of prawn, with respect to time, under three different anesthetics applied at different concentrations.*

mortality. Morgan et al. (2001) evaluated clove oil as an anesthetic for use on three Pacific coast crab species: dungeness crab *Cancer magister*; hairy shore crab *Hemigrapsus oregonensis*; and northern kelp crab *Pugettia producta*. Clove oil was the most effective in kelp crab with induction times ranging from 54 to 2 min at concentrations of 15 to 250 mg/L. Dungeness crab had induction times ranging from 68 to 16 min at 500 to 1,500 mg/L, and shore crab demonstrated the longest induction times (188 to 87 min) and required the highest concentrations (1,000 to 3,000 mg/L) to achieve immobilization. There appears to be significant variability within Crustacea on the induction times and concentrations required for anesthesia using clove oil. This may indicate that research should be conducted for each species before use. Although clove oil proved to be an effective anesthetic for use with prawn, it currently is not approved by the FDA for use on food fish or crustaceans in the United States. In addition, the likelihood of clove oil being approved for use as an anesthetic is poor due to a lack of sponsorship, high composition variability between sources, and the carcinogenic nature of some constituents (isomers) (FDA 2002).

Aqui-S™ was found to be effective on freshwater prawn as a mild sedative at a rate of 100 mg/L. When the prawn were treated with Aqui-S™ at 200–300 mg/L, significant mortality was observed during recovery. However, the application rates used in this study were very high compared to those recommended by the manufacturer for use on fish (10–25 mg/L). It appears that the safety margin is much greater for clove oil than for Aqui-S™ in prawn. However, Gardner (1997) recommended 125 mg/L clove oil or 500 mg/L Aqui-S™ as effective treatments for the humane killing of the Australian giant crab *Pseudocarcinus gigas* implying that greater concentrations of Aqui-S™ were required to kill *P. gigas*. Apparently, the efficiency of Aqui-S™ may vary between species of crustaceans, as it does in fishes. If a partial loss of equilibrium is found to be a suitable level of anesthesia, Aqui-S™ applied at 100 mg/L may prove to be effective for transport conditions. Clove oil and Aqui-S™ each have desirable traits for use in aquaculture: ease of use (relative to carbon dioxide) and calm induction to anesthesia. However, Aqui-S™ has the greatest potential for being approved for use in the U.S. because it is currently undergoing the NADA process.

Based on the criteria of this experiment, quinaldine was not as effective as clove oil or Aqui-S™. In quinaldine treatments, total loss of equilibrium (rank 2) was not achieved in any

of the levels tested (100–300 mg/L). Schoettger and Steucke (1972) reported that rainbow trout *Oncorhynchus mykiss* exposed to quinaldine, even at a state of total loss of equilibrium (stage 2 in this study), retained a strong reflex response to being touched. Summerfelt and Smith (1990) listed a poor suppression of reflex actions by anesthetized animals and irritability to mucous membranes as some of the major drawbacks of quinaldine. In the present study, prawn exposed to quinaldine exhibited muscle spasms and attempted to escape the tank. In contrast, prawn exposed to clove oil and Aqui-S exhibited a much calmer response to anesthesia. Munday and Wilson (1997) reported that a coral reef fish *Pomacentrus amboinensis* exposed to quinaldine swam rapidly and attempted to jump out of the tank when exposed to the bath treatment. If total loss of equilibrium is not necessary, 100–200 mg/L of quinaldine may be an effective anesthetic for prawn. Quinaldine is not approved by the FDA for use on food fish in the U.S. and is reported to be carcinogenic (Summerfelt and Smith 1990). However, quinaldine is legal for use in some countries, and may be useful for sedating brood prawn in hatchery operations.

The properties required of an anesthetic vary with the procedure or objective. In this study the desired effect was total sedation, primarily to compare the relative effectiveness and margins of safety for the different anesthetics. A quick induction of anesthesia is desirable in most cases. In practice, a total loss of equilibrium may not be desirable in transport because overcrowding of sedated prawn at the bottom of the transport tank could result in asphyxiation. Under transport conditions, the sedation level 1 used in this study would likely be more appropriate. Therefore, clove oil or Aqui-S™ applied at 100 mg/L may be the best options when transporting prawn. The results of this experiment will be used to determine effectiveness of different concentrations of these anesthetics in improving prawn survival under actual transport trials. However, at this time there is no chemical anesthetic that is approved by the FDA for use with 0-withdrawal in the U.S. For producers to use anesthetics for transporting live prawn to market a 0-withdrawal anesthetic approved by FDA under NADA will be necessary.

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