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Use of Injectable Eugenol for Euthanasia and Anesthesia of American Lobsters (Homarus Americanus) and Similar Species

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Abstract

Crustaceans are economically and ecologically significant, but current treatment and diagnostic protocols for crustacean health are limited. According to standards given by the American Veterinary Medical Association there are no current methods of euthanizing lobsters, nor are there effective methods of quick release anesthesia. The objective of this research demonstrated that eugenol by direct injection is a safe, efficient and reliable method for euthanizing or anesthetizing crustaceans. Anesthetic levels were determined by behavior responses, death was determined by a lack of response to stimuli. The results presented here suggest eugenol can be used as a euthanizing agent for American lobsters (*Homarus americanus*) and green crabs (*Carcinus maenas*) injected into the sinusoidal circulatory system at a dose of 7µl/g for lobsters and 10µl/g for crabs. Crabs and lobsters were anesthetized by a dose of 0.15 µl/g dissolved in a solution of 70% ethanol and sterile sea water injected into the pericardial sac.

Euthanasia and anesthesia methods for *H. americanus* and similar species

Introduction:

The American lobster (*Homarus americanus*) is a commercially important fishery species in an area that extends from Canada to the coast of New England. In 2010 Maine fishermen caught 96 million pounds of lobsters worth $318 million dollars (Maine DMR 2011).

Lobsters are invertebrates and have a neural system with pain receptors (Elwood *et al.* 2009). Evidence for pain receptors suggests the need for humane methods for anesthetizing and euthanizing crustaceans. However, lobsters and other crustaceans generally don't respond to conventional emersion fish anesthetics such as MS-222 (tricane methanesulfonate) or quinaldine sulfate (Coyle *et al.* 2005). Current euthanasia methods include freezing, direct blunt force to the rostrum or injection of potassium chloride. These methods are not considered safe or humane according to standards given by the American Veterinary Medical Association (2007; Battsion *et al*. 2000).

Developing an effective and safe anesthetic which can be used in the laboratory as well as in the field is important in order to conduct experiments to increase general knowledge of the species and to provide proper management for the fisheries. Shell disease has been damaging the lobster fisheries since the early 1990's and has become increasingly common with increasing sea water temperature (Smolowitz *et al.* 2005; Glenn & Pugh 2006). To improve the research methods used on lobsters including important research related to shell disease a standard method for the anesthesia of lobsters should be developed. A quick release anesthetic would reduce stress of handling for lobsters, and researchers, and could be used to reduce stress on lobsters during shipping.

Euthanasia and anesthesia methods for *H. americanus* and similar species

The primary focus is a method for euthanasia and anesthesia of lobsters because of their commercial importance, but the method was also tested with other decopod crustaceans. There is a close taxonomical relationship within the same phylogenetical order between *H. americanus* and other decapods found in Rhode Island waters, such as the green crab *Carcinus maenas,* and the rock crab, *Cancer irroratus* (Hughes & Matthiessen, 1962). Due to the close taxonomical relationship between the crabs used in this study and *H. americanus* the reaction to eugenol was hoped to be similar.

The growth pattern of lobsters and similar species create a unique problem for calculating a dose relative to the muscle mass. The growth of lobsters is limited by their exoskeleton and they must undergo ecdysis in order to grow (Chang, 1995). Between molts the size of the exoskeleton remains the same, but during ecdysis the total body mass and size increase as a result of the intake of water (Radhakrishnan & Vijayakumaran, 1984; Mykles, 1980). Therefore, the relationship between size, mass and amount of tissue vary with the molt cycle (Mykles, 1980).

Lobsters at market size molt approximately once per year (Laufer *et al*. 2005). Ecdysis, the process of molting, is primarily controlled by seawater temperature, photoperiod and hormones such as ecdysone produced by the lobster (Quackenbush, 1994). Ecdysone usually peaks in late June or early July and is directly related to the time of ecdysis (Laufer *et al*. 2005). There are also other physiological changes related to molt cycle such as gonad development, which is also regulated by ecdysone and other hormones. Mating occurs when freshly molted females release pheromones. After molting season the gonadal tissue is reduced by biological atrophy (Hughes & Matthiessen, 1962).

Eugenol has been used as a dental pain reliever in humans and has anesthetic properties in other animals. The fish anesthetic Aqui-S® made primarily of isoeugenol has been used as a common fish emersion anesthetic and euthanasia agent (Meinertz *et al.* 2006). Preliminary research showed that eugenol caused some cancer in test animals. Isoeugenol, which is an isomer characterized by a change in the placement of the double bond on the methyl tail of the phenol group, was thought to be safer (Figure 1) (National Toxicology Program 1983; Center for Veterinary Medicine, 2007). Recently it has been determined that isoeugenol was the impurity in eugenol which was responsible for causing cancer in test animals (National Toxicology Program 2010). Eugenol is an ecological safe compound. It is the primary component of clove oil and requires no withdrawal time after animals have been exposed.

Aqui-SE®, a new product containing eugenol without the impurity of isoeugenol, is currently being investigated for use as a quick release fish anesthetic in the United States (United States Fish and Wildlife Services 2011). Lobsters have had a moderate anesthetic response when eugenol was added directly to the water but the dose required is high at 75-100 mg/l compared to the dose used for most fish, 14-34 mg/l (Waterstrat & Pinkham 2005; Meinertz *et al.* 2006). Using eugenol as an emersion anesthetic also is not reliable for each treatment (Smolowtiz, personal communication). By using direct injection the high dose required for anesthesia of crustaceans can be reduced and the aroma, which some people are sensitive to, can be controlled more readily. Eugenol is only slightly miscible in water but will dissolve more readily when mixed with 70% ethanol and then it can be diluted with cold water (Hajek *et al*. 2009; Saydmohammed & Pal, 2009).

Euthanasia and anesthesia methods for *H. americanus* and similar species

The objective of this research was to determine the anesthetic ability of eugenol injected into the sinusoidal fluid of decapod crustaceans. The expectation was that it would have a faster and more direct anesthetic result than other methods. Eugenol was also tested here to determine if high doses would be a safe and effective euthanizing agent for lobsters and crabs.

Materials and Methods:

Euthanasia methods

Pre-injection:

American lobsters (*Homarus americanus*), rock crabs (*Cancer irroratus*), and green crabs (*Carcinus maenas*) were collected by SCUBA or metal lobster traps intermittently during the experiment from Narragansett Bay, Rhode Island between June 2010 and August 2011. Length, width, height, sex and mass were recorded for each animal (Figure 2, Table 1). The animals were identified by attaching a cable tie loosely around the cheliped. Lobsters had their claws restricted by rubber bands which were removed prior to injection in order to assess behavior. The animals were acclimated for a period of 3-5 days before injection during which health was assessed by behavior observations including response to feeding and interactions between animals. During the acclimation period each animal was observed to determine their pretreatment response to lateral (lobsters) or dorsal (crabs) recumbency. They were fed blue mussels (*Mytilus edulis*) while in captivity.

The animals were stored in 95 liter tanks with a semi-recirculating system. Ambient unfiltered water constantly flowed into the tanks from an intake in Mount Hope

Bay, Bristol Rhode Island. The temperature was controlled by Teco chillers (model TR-15) set at 12°C or 18°C. Water quality was monitored by measuring salinity, dissolved oxygen, ammonia, nitrate, nitrite, and pH.

Three crabs and two lobsters were dissected to determine the least destructive injection path for delivery of the anesthetic solution into the sinusoidal circulatory system, specifically the pericardial sac surrounding the heart (Figure 3&4). One lobster and two crabs were then injected with a euthanizing dose of eugenol (10µl/g) dyed with neutral red to visualize the path of the injected solution (Figure 5&6).

Behavior Evaluation:

The post injection behavior response evaluation parameters were adopted from Waterstrat & Pinkham (2005). Three behavioral categories were evaluated: loss of aggression, immobilization, and loss of equilibrium. Aggressive behavior was defined as raising of the claws and body in a protective stance, backing away with claws raised or attacking when provoked. Mobilization was defined as walking, standing, or noticeable movement of the limbs or tail. Equilibrium was measured as the animal's ability to right itself from recumbency. Each behavior was graded on a scale between 0-3 to determine the level of response with 0 being no effect and three being the maximum response (Table 2). Death was determined by the lack of response to stimuli such as touching of the eye stalk, and by no movement of the mouth parts for three hours. Recovery was defined as when the animal exhibited normal behavior. Side effects evident after recovery consisted of animals with normal behavior excepted for lack of movement in specific limbs or the tail

Euthanasia and anesthesia methods for *H. americanus* and similar species

Injection:

Eugenol (2-Methoxy-4-(2-propenyl) phenol-99%, fisher scientific Cat#AC11911-1000) was used without further purification to determine the minimum euthanizing dose for *H. americanas* and similar species. The dose used was recorded as the amount of eugenol/total mass of the animal. To determine the minimum effective euthanizing dose of eugenol the following doses were tested on crabs; 20µl eugenol/g of body mass (21.2mg of eugenol/g of body mass), 10µl/g (10.6mg/g) or 7µl/g (7.42mg/g). Lobsters were injected with only a dose of 7µl/g (7.42mg/g).

The eugenol was injected directly to the pericardial sac using a syringe with a needle appropriate for that animal. Crabs with a carapace over 80 mm wide and 60 mm long were injected using a 3 ml syringe with a 22-gage, 25 mm long needle. For smaller crabs a 1 ml syringe was used with a 25-gage, 16 mm long needle. For legal sized lobsters (carapace length 84.1-127mm) a 3 ml syringe with a 22-gage, 25 mm long needle was used. The volume of eugenol used was calculated by multiplying the desired dose by the wet mass of the animal. The eugenol was drawn directly into the syringe and injected into the pericardial sac through the arthrodial membrane at the joint between the cephalothorax and abdomen on the dorsal surface (Figure 7&8). The syringe was angled toward the cervical groove (visible on the dorsal surface of the cephalothorax) and injected at a rate of 0.5 ml/sec.

Post injection:

Confirmation of Euthanasia:

Observations were initially made after injection then one minute after injection the animals were returned to the observation tank. Further evaluations were conducted every five minutes until the animal appeared to be dead. Death was determined when the animals reached total loss of movement (A3, M3, E3) and time until death was recorded as time from injection until these non-responsive behavior levels were identified. Observations post-apparent death continued for at least three hours at which point animals were frozen for later disposal.

Sampling of Tissues:

At each euthanizing dose, tissues were collected from two green crabs and one lobster to be processed for histological evaluations in order to evaluate the effect of the eugenol. The effect of the eugenol injection was compared to controls consisting of two green crabs and one lobster which had no injection prior to the necropsy; these were processed for histological evaluation. The tissue sampling for the necropsy was conducted at three minutes after injection for euthanizing doses. The necropsies were conducted by removing the carapace and making observations on the condition of the tissues. Then tissue samples from heart, gonad, neural, hepatopancreas, gill and muscle near the injection site were removed for histological evaluation. The tissues were fixed in 10% formalin in seawater. The gill and injection site were decalcified following the methods of Luna (1992) using the formic acid-sodium citrate method. The tissues were stored in 70% ethanol until trimmed and placed in histology cassettes stored in 70%

ethanol to await further processing. The tissues were embedded in paraffin. Section cut and stained with hematoxylin and eosin stains at an outside laboratory (Mass Histology Services, Inc., Worcester, MA).

Anesthesia methods

Pre-injection:

The methods used to determine the appropriate eugenol dose for anesthesia of *H. americanus* and similar species were similar to the euthanasia methods. The animals were processed the same as for the euthanasia methods until the injection of eugenol.

Injection:

For anesthetic doses of crabs and lobsters the following doses were tested; 0.2µl of eugenol/gram of animal mass (0.212mg/g), 15µl/g (0.159mg/g) or 0.10µl/g (0.106mg/g). The desired dosage was multiplied by the total body mass to determine the volume of eugenol. The eugenol was added to an eppendorf tube using a micropipette and was dissolved with twice the volume of 70% ethanol. This solution was further diluted, depending on the size of the animal, with autoclaved-filtered (0.2µm) sea water. The total volume of solution was based on the size of the animal. Larger crabs that were at least 80 mm wide and 60 mm long were injected with 0.5 ml of total solution and smaller ones with 0.3 ml. Legal sized lobsters were injected with 1.5 ml of the anesthesia solution. Size of the syringe and needle used were as describe for the euthanasia methods.

Needle injection methods used for anesthesia of crabs was similar to those described for euthanasia methods except, the needle was angled towards the carapace to reduced tissue. To reduce the risk of infection the injection site was cleaned with 70% ethanol before an injection for anesthesia. To inject an anesthetic dose of eugenol into a lobster it was held on its side in lateral recumbancy, and then the needle was placed into the arthrodial membrane at the base of the third walking leg. The needle was angled straight towards the top of the carapace between the body cavity and the gills (Figure 9). The needle was inserted to at least half the height of the carapace, which for legal sized lobsters, was the full length of the needle (22 gage, 25 mm long needle)

Post injection:

Behavior response evaluation methods were the same as described for the euthanasia methods. The animals were determined to be anesthetized when they reached an aggressive level of 3, movement level of 2.5, and equilibrium level of 3 (A3, M2.5, E3) which resulted in no aggressive behavior, showed no limb movement, the loss of any apparent sense of equilibrium but still showed eye or mouth movement in response to stimuli (Table 2). "Light anesthesia" was classified as a moderate behavior response but not full anesthesia. The animals were observed every five minutes after the injection until they had become anesthetized (A3, M2.5, E3). They were observed every 10 minutes until they began to recover. If no movement or response was identified for three hours the animal was considered dead and placed in the freezer for disposal.

After the animals recovered from anesthetic doses they were observed daily for at least a week and fed blue mussels (*Mytilus edulis*) twice a week. After a week of observation the result of the anesthesia were classified as full recovery, recovery with side effects (paralysis), no effect (not anesthetized) or mortality.

At each anesthetic dose (0.2µl/g, 15µl/g and 0.10µl/g) tissues were collected from two green crabs and one lobster, were processed for histological evaluations in order to evaluate the effect of the eugenol injection. The tissue sampling was conducted at 30 minutes after injection and tissues were processed as described in the euthanasia methods. Necropsies were also conducted on two green crabs and one lobster injected with a suspension of ethanol and sea water only in order to determine the effect of the needle and the carrier on the tissues.

Statistical analysis

The difference in the time until immobile (A3, M2.5, E3) and time until euthanized (A3, M3, E3) were compared between temperatures, species, and doses using a two way-anova. The reaction to anesthetic doses was compared using percentages of success rate, recovery with side effects, no effect and mortality. The student t-test was also used to compare the time until immobile and the time under heavy anesthesia. For all tests a confidence at ≤ 0.05 was used.

Results:

Euthanasia

Eugenol injected into the pericardial sac of American lobster (*Homarus americanus*), rock crabs (*Cancer irroratus*), and green crabs (*Carcinus maenas*) functioned as a euthanizing agent at each dose tested (20 µl/g, 10 µl/g and 7 µl/g). The reaction time varied depending on the water temperature at which the animals were held, the season of the year, and the dose. No animals showed any movement or other sign of life during the three hour period after they were identified as dead.

H. americanus kept at 18° injected with a dose of 7µl/g were immobile at 4.8 ±1 min and appeared dead in 32 ±10 min. At 12°C the same dose of 7µl/g took almost double the time for a response, they were immobile in 9 ±1.9 minutes and appeared to be dead 62 ±1.5 min after injection (Figure 10 &11).

C. maenas injected with 20µl/g at 18°C were immobile (A3, M2.5, E3) in an average of 1.2 ±0.1 min. After they were identified as immobile there was movement of the eyes and mouth parts in response to stimulus, until they appeared dead 1.6 ±0.1 min after injection. When the dose was reduced to 10µl/g they were immobile after 2.4 ±0.2 min and appeared dead at 9.9 ±0.8 min. The crabs kept at 18°C and injected with 7µl/g became immobile after 2.5 ±0.4 min and appeared dead 32 ±3.1min after injection (Figure 10 &11).

The *C. maenas* which were kept at 12°C and injected with a dose of 20µl/g were immobile in 1.2 ±0.1 min and appeared dead in 12.8 ±4.9 min. *C. maenas* injected with 10µl/g at 12°C during time 1 were immobile after 10 min and appeared dead in 47 ±3.5 min (Figure 10 &11).

C. irroratus which were kept at 18°C and injected with 20µl/g were immobile in an average of 1.1 ±0.1 min, they appeared dead 2.0 ±0.2 min after injection. When the dose was reduced to 10µl/g the crabs were immobile after 1.1±0.1 min and appeared dead at 35 ±4.9 min. The crabs kept at 18°C and injected with 7µl/g became immobile after 2 min and were appeared dead 26 ±1 min after injection (Figure 10 &11).

C. irroratus kept at 12°C were injected with a dose 20µl/g became immobile in an average of 1.5 ±0.1 min, they appeared dead 19.6 ±3.2 min after injection. When the dose was reduced to 10µl/g the crabs were immobile after 3.3 ±0.5 min and appeared dead at 99 ±9.8 min. Crabs kept at 12°C injected with a dose of 7µl/g took an average of 10 min for them to become immobile and they appeared dead 100 ±2.8 min after injection (Figure 10 &11).

Anesthesia

A difference was seen in reaction to anesthetic doses of eugenol in the second half of the summer (beginning in mid-July for both 2010 and 2011). Therefore the results for anesthesia have been separated into two groups based on the months in which the animals were injected. The date separating the two groups was July 15th. This change correlated with the time usually identified as a molting date after which there is normal atrophy of the gonad tissue and crustaceans undergo other physiological post-molt changes (Quackenbush, 1994). The two groups were designated time 1 and time 2. Time 1 included the animals which were injected from the spring until July 15th. Time 2 included the animals injected after July 15th until October. No animals were injected between October and March.

Time 1

An anesthetic dose of 0.2 µl/g resulted in anesthetization for 8 out of 9 *C. maenas*. However, one crab showed no response to the injection. The 8 affected crabs injected with 0.2µl/g became immobile (A3, M2.5, E3) in 11.5 ±2.2 min after injection. They began to recover at an average time of 80 ±12 min after injection (Figure 12). All the crabs recovered fully except for one crab which showed paralysis post injection at the fourth walking leg (Table 4).

Four *H. americanas* were injected with 0.2µl/g during time 1, one of the lobsters showed no response to the injection of eugenol. Three were lightly anesthetized (A2, M1, E2) from 15 minutes after the injection until 45 minutes after the injection when they progressed to exhibit deep anesthesia until they recovered 94 ±28 min after the injection (Figure 12). One of the anesthetized lobsters showed paralysis of the third walking leg (Table 3).

One lobster was injected with 0.1µl/g during time 1 and never reached full anesthesia; it did exhibit aggressive behavior and had trouble walking (A1.M1,E3), but fully recovered.

Time 2

Eight *C. maenas* were injected with 0.2µl/g in time 2 (Table 4). All of these crabs died within 1 day of injection showing no signs of recovery from the anesthetic. One lobster, injected with 0.2µl/g appeared to be dead three hours after injection (Table 3).

Five *C. maenas* were injected with 0.15µl/g and were anesthetized after 15 ±2.6 minutes then fully recovered after 67 ±5.1 min. Three lobsters were kept at 18°C and

injected at a dose of 0.15µl/g and became anesthetized after 11.6 ±3.3 min and recovered after 70 ±5 min (Figure 12). Two *H. americanaus* were kept at 12°C and injected with 0.15µl/g and were immobile after 10 min they began to recover after 25±5 min. Three of the lobsters which where anesthetized at 0.15µl/g had paralysis of either the tail or a walking leg post-anesthetic recovery (Table 3). After the one week observation period the paralysis was still present and the animals were euthanized.

Three *C. maenas* injected with 0.1µl/g became anesthetized after 3 min and recovered one hour after injection (Figure 12). One of the crabs showed paralysis of the abdomen, from post-injection recovery to one week after injection. After the one week observation period it was euthanized.

Four *H. americanus* held at 12°C were injected with 0.10µl/g became anesthetized after 13.7 ±2.4 min and recovered after 70 ±11 min (Figure 12). Three of these lobsters showed paralysis as they recovered from anesthesia (Table 3). These three lobsters were held for one month in the observation tank to determine if they were able to recover from paralysis overtime. One month post-injection they had all regained full movement.

The control animals injected with only the ethanol/sea water mixture and no eugenol had slight behavior changes (A2, M1, E2) but fully recovered after 20 min and remained healthy during the one week observation period.

Histological Results

During the tissue sampling no tissue damage was identified grossly. Histological examination of tissues collected after injections showed tissue damage related to the injection. Major damage to the tissues was noted at the euthanizing doses of 20µl/g. The majority of the tissue damage was severe multifocal peracute necrosis of muscle bundles with fragmentation and vacuolation of muscle fibers. Swelling and edema was seen in the connective tissue surrounding the necrotic muscle (Figure 13). The digestive gland and gonadal tissue adjacent to the injection site also was affected. In many of the animals injected with 20µl/g, severe multifocal muscle necrosis was seen in the cardiac muscle bundles (Figure 14). When the euthanizing dose was reduced to 10µl/g or 7µl/g a similar effect of the eugenol on the tissues was seen, but was markedly reduced.

At anesthetic doses (0.2µl/g) minor tissue damage was noted histologically. The majority of the damage occurred in muscle adjacent to where the eugenol was injected and appeared to be strongly associated with damage from the needle path (Figure 15). Tissue samples taken a week after injection of 0.2µl/g showed evidence of reparative inflammatory response in muscle and connective tissue disrupted by the needle insertion.

In order to understand the reparative inflammatory response tissue samples were taken one month after injection from two lobsters. These two lobsters were anesthetized with 0.10 µl/g and exhibited post-injection paralysis of two walking legs near the injection site. The animals no longer exhibited paralysis when then tissue samples were taken. Histological evaluation of the tissues showed chronic reparative hemocytic inflammation and regeneration of affected muscle bundles (Figure 16). A pseudomembrane, produced by migrating hemocytes formed a “wall” underneath the

damaged tissue at the injection site (Smolowitz *et al.* 2005). This pseudomembrane was internally lined by a newly produced inflammatory membrane which isolated the necrotic tissue to the surface of the wound. Isolating the necrotic tissue to the surface would have allowed it to be shed at the lobsters next molt (Figure 15).

Discussion

The evidence for pain receptors suggests that humane methods for anesthetizing and euthanizing crustaceans should be pursued both for research and to improve the shipping and handling of lobsters. Direct injection of eugenol into the pericardial sac of crabs and lobsters will result in anesthesia, and at higher doses euthanasia. Problems which have occurred with anesthetic dosages are paralysis of limbs or tail, and inconsistency with the response to anesthetic doses.

There was a seasonal change identified in the anesthetic response to eugenol. This change was noted both in 2010 and 2011. After the predicted molt season green crabs and lobsters died at doses which succeeded as anesthesia during the spring and early summer. Specifically, during time 1 (the spring and early part of the summer) a dose of 0.2µl/g resulted in a heavy anesthesia and full anesthetic recovery in crabs and lobsters. However in time 2 the same dose resulted in the death of eight green crabs and one lobster. During time 2, a dose of 0.1µl/g resulted in anesthesia of green crabs and lobsters. The changes in reaction to eugenol could be a result of physiological changes. During the spring and early summer the gonads in sexually active decapods produced mature eggs and sperm in a response to the photoperiod and water temperature in order to prepare for mating season (Quackenbush, 1994). The hydrophobic properties of eugenol

make it easily absorbed by tissues with high lipid concentrations such as the gonads. The physical atrophy of the gonads at the end of the summer results in the loss of the gonadal sink. This results in the need for a lower amount of anesthetizing dose as well as a lower euthanizing dose. Interestingly this seasonal effect was not observed in rock crabs.

Variations in the anesthetic reactions to eugenol at specific doses were also seen by Coyle *et al.* (2005) when eugenol was evaluated as an emersion anesthetic for the freshwater prawn (*Macrobrachium rosenbergii*). For this experiment the doses were based on the total mass of the animal. This does not take into account the difference of body mass compared to shell mass during the molt cycle or loss of appendages. This experiment did not find an efficient method to calculate the muscle mass of a live decapod to calculate an anesthetic dose of eugenol proportional to muscle mass not total body mass.

During this study some crabs and lobsters recovered from anesthesia but still had weakness or paralysis of a leg or the tail after one week of observation. Histological evaluation of the tissues showed that the eugenol damaged the tissues with the majority of the damage occurring in adjacent muscle. The inflammatory response occurring in muscle after one week of injection suggested that the paralysis was not permanent and that the animals would be able to recover. Observation of lobsters with paralysis post-injection showed that lobsters did regain movement of the affected muscle after approximately one month.

The higher doses reduce the time until the animals were unresponsive, yet some animals underwent autotomy (self amputation) near the injection site. The lower euthanizing dose reduces the stress on the animals by inducing anesthesia in an average

of five min after which the animals were not responsive to pain. During the period after which the animals were immobile the only movement observed was slight response to major stimuli. Apparent death in this study was determined by behavior response. In multicellular organisms their cells do not all die at the same time. In most cases the recorded time of death is likely later than the actual time and movement *perimortem* was a result of automatic response by stimulated the cells (Anderson & Macleod, 1930).

In this study the concentration of residual eugenol in the tissue was not determined in the injected animals. Other studies in which crustaceans were exposed to eugenol were able to reduce levels of eugenol from the treatment concentration (33µl/g) to below 11.3µl/g after 12 hours and after 24 hours the concentrations were below the detection limit of 0.377 µl/g (Saydmohammed & Pal 2009). The amount of eugenol required for euthanasia and anesthesia by direct injection is significantly lower than the amount absorbed by tissues during emersion treatments (Kildea *et al.* 2004). Eugenol is currently an approved oral anesthetic in humans and the expected residual levels are predicted to be undetectable and safe for consumption in treated animals.

Eugenol injected directly into the pericardial sac of lobsters will serve as a euthanizing agent for *H. americanus* and similar species at a dose of 7.0µl/g for lobsters and 10µl/g for crabs. After the eugenol is injected the animals became docile and unresponsive within minutes then die. Eugenol can also be used as an anesthetic by injection at a dose of 0.15µl/g for crabs and lobsters with an average anesthetic duration of 50 min. Once eugenol is approved as an anesthetic in the United States it can allow for a quick release crustacean anesthetic for field studies, and laboratory research as well as provide a humane euthanizing method while preserving tissues.

Referemces:

American Veterinary Medical Association 2007. Report of the AVMA panel on euthanasia. J Am Vet Med Assoc 218:669–696.

Anderson, I., and Macleod, J. 1930. CLIV. The glycogen of mammalian muscle and its behavior after death. *Biochem.* 14:1407-1420

Battison, A., MacMilan, R., MacKenzie, A., Rose, P., Cawthorn, R., & Horney, B. 2000. Use of injectable potassium chloride for euthanasia of American lobsters (*Homarus americanus). Amer Assoc Lab Anim Sci.* 50:545-550.

Center for Veterinary Medicine. 2007. Guidance for Industry: Concerns related to the use of clove oil as an anesthetic of fish. US Department of Health and Human Services.

Chang, E. 1995. Physiological and biochemical changes during the molt cycle in decapods crustacean: an overview. *J Exp Mar Biol Ecol.* 193:1-14.

Coyle, S., Dasgupta, S., Tidwell, J., Deavers, T., Bright, L., & Yasharian, D. 2005. Comparative efficacy of anesthetics for the freshwater prawn *Macrobrachium rosenbergii. J. World Aquacult. Soc.* 36:282-290.

Elwood, R., Barr, S., & Patterson, L. 2009. Pain and stress in crustaceans? *Apl Ani Behav Sci.* 118:128-136.

Glenn, R. & Pugh, T. 2006. Epizootic shell disease in American lobster (*Homarus americanus)* in Massachusetts coastal waters: interactions of temperature, maturity and intermolt duration. *J. Crustacean Bil.* 26:639-645.

Hajek, G., Choczewski, M., Dziaman, R.,& Klyszejko, B. 2009. Evaluation of immobilizing methods for the 19Chinese mitten crab *Eriocheir sinensis* (Milne-Edwards). *Electronic journal of polish agricultural universities.* 12:

Hughes, J., Matthiessen, G. 1962. Observations on the Biology of the American Lobsters, *Homarus americanus. Limnol Oceanogr.* 7:414-421.

Kildea, M., Allan, G., & Kearney, R. 2004. Accumulation and clearance of the anesthetics clove oil and AQUI-S™ from the edible tissues of silver perch (*Bidyanus bidyanus*). *Aquaculture*. 232:265-277.

Laufer, H., Demir, N., Biggers, W.B. 2005. Response of the American Lobster to the stress of shell disease. *J. Shellfish res.* 24:757-760.

Luna, L. 1992. Histoplathologic methods and color atlas of special stains and tissue artifacts. Johnson Printers, IL.

Maine Department of Marine Resources (DMR). Feb 17, 2011. Historical Maine Lobster landings.

Meinertz, J., Greseth S., Schreier T., Bernardy, J. & Gingerich, W. 2006. Isoeugenol concentrations in rainbow trout (*Oncorhynchus mykiss*) skin-on fillet tissue after exposure to AQUI-S™ at different temperatures, durations, and concentrations. *Aquaculture* 254:347-354.

Mykles, D. 1980. The mechanism of fluid absorption at ecdysis in the American lobster, *Homarus americanus. Exp Biol.* 84:89-101.

National Toxicology Program. 1983. Carcinogenesis studies of eugenol in F344/N rats and $B6C3F_1$ Mice (feed studies). National institute of health 84-1779. NTP Technical report series no. 223: 1-223.

National Toxicology Program. 2010. Carcinogenesis studies of isoeugenol in F344/N rats and $B6C3F_1$ Mice (gavage studies). National Institute of Health 10-5892. NTP Technical report series no. 551: 1-180.

Quackenbush, S. 1994. Lobster Reproduction: a review. *Crustaceana.*67:82-94

Radhakrishnan, E., and Vijaykumaran. 1984. Effects of eyestalk ablation in spiny lobsters (*Panulius homaus)* on molting and growth *Indian J Fish* 31: 130-147.

Saydmohammed, M., & Pal, A. 2009. Anethetic effect of eugenol and methol on handling stress in *Macrobrachium rosenbergii. Aquaculture.* 298:162-7

Smolowitz, R., Christoserdov, A. & Hsu, A. 2005. A description of the pathology of epizootic shell disease in the American lobster, *Homarus americanus*, Milne Edwards 1837. *J. Shellfish Res.* 24: 749-56.

United States Fish and Wildlife Service. 2011. Fact sheet: Aqui-S® EINAD 11-741. The aquatic animal drug approval partnership program.

Waterstrat, P., & Pinkham, R. 2005 Evaluation of Eugenol as an Anesthetic for the American Lobster *Homarus americanus. J. wrld aqua soc.* 36.3:420-424.

Tables and Figures

Table 1- Average size and sex of animals used for this study. Carapace length was recorded for lobsters. For crabs carapace width was recorded.

		Sample size	Average Carapace Length or width (mm)	Average mass (g)	Percent males	Percent Females
Euthanasia	Lobsters	9	445 (±35)	82 (±2.2)	89%	11%
	Green Crabs	44	77.8 (±4.3)	63.8 (±1.3)	69%	31%
	Rock Crabs	49	202 (±6.4)	100 (±1.2)	100%	0%
Anesthesia	Lobsters	16	442 (±29)	81.2(±2.2)	85%	15%
	Green Crabs	24	57 (±3.7)	58 (±1.4)	33%	67%
	Rock Crabs	4	77.8 (±2.2)	75 (±1.1)	100%	0%

Table 2-Scale of behavioral response rating for decopod crustacean injected with eugenol

	Level	Behavior Changes
Aggression	0	No notable changes
	1	Moderate aggression
	2	Slow and weak aggressive behavior
	3	Total loss of aggression
Mobility	0	No notable changes
	1	Able to walk but slow and clumsy
	2	Only able to move/twitch the limbs; can't support weight
	2.5	No limb movement; eye/mouth movement to stimuli
	3	Total loss of movement
Equilibrium	0	No notable changes
	1	Moderate balance; Struggled to recover from recumbency
	2	Stumbling and falling Can't recover from recumbency
	3	No sense of equilibrium

Table 3- Results for *H. americanus* after one week observation period post-injection of anesthetic doses of eugenol. One animal injected at 0.2µl/g showed no effect

Dose	Season	n	Recovered	Recovered with side effects
0.2µl/g	Time 1	5	100%	20%
0.2µl/g	Time 2	1	0%	NA
0.15µl/g	Time 2	5	100%	60%
0.1µl/g	Time 1	1	100%	0%
0.1µl/g	Time 2	4	100%	75%

Table 4- Results for *C. maenas* after one week observation period post-injection of anesthetic doses of eugenol

Dose	Season	n	Recovered	Recovered with side effects
0.2µl/g	Time 1	8	100%	12%
0.2µl/g	Time 2	8	0%	NA
0.15µl/g	Time 2	5	100%	0%
0.1µl/g	Time 2	3	100%	33%

Table 5- Results for *C. irroratus* after one week observation period post-injection of anesthetic doses of eugenol

Dose	Season	n	Recovered	Recovered with side effects
0.2µl/g	Time 1	2	100%	0%
0.2µl/g	Time 2	1	100%	0%
0.15µl/g	Time 2	1	100%	0%

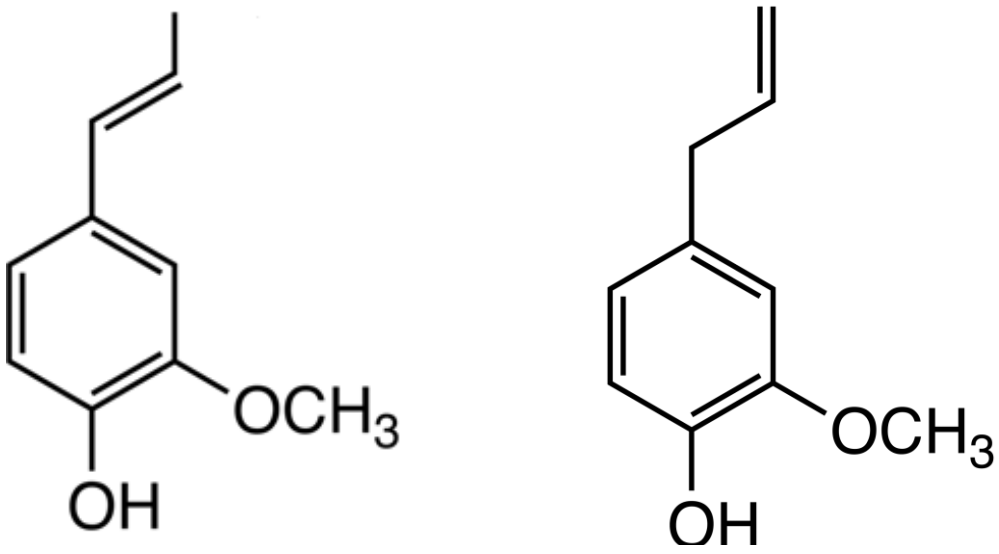

Figure 1- Structural difference between isoeugenol (2-Methoxy-4-(1-propenyl)phenol) and eugenol (2-Methoxy-4-(2-propenyl)phenol

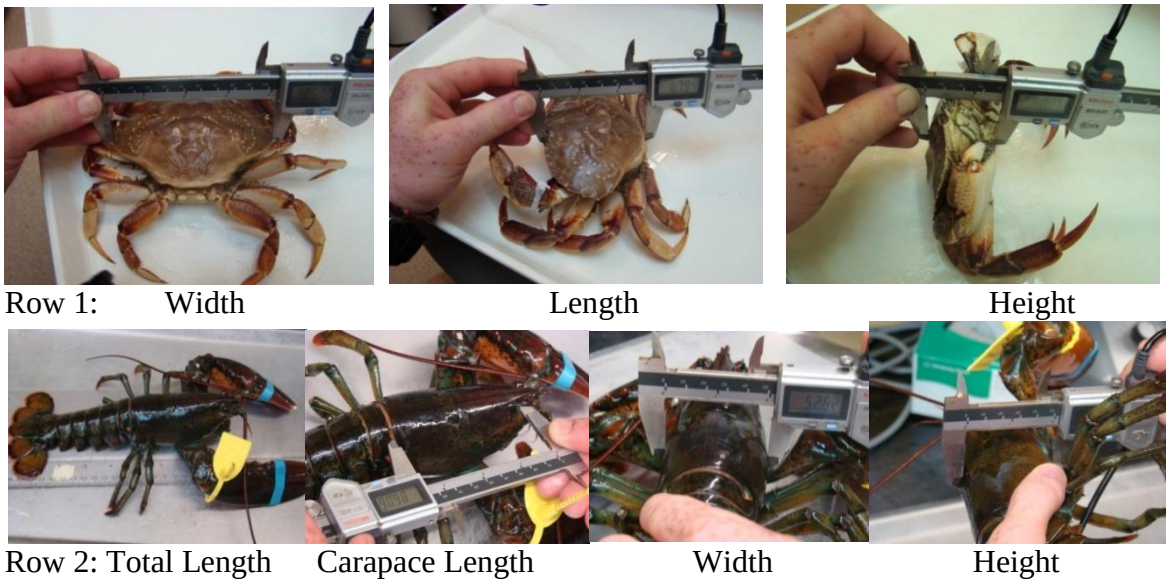

Figure 2- Visual representative of the methods used to measure *C. maenas* and *C. irroratus* (row 1) and *H. americanus* (row 2).

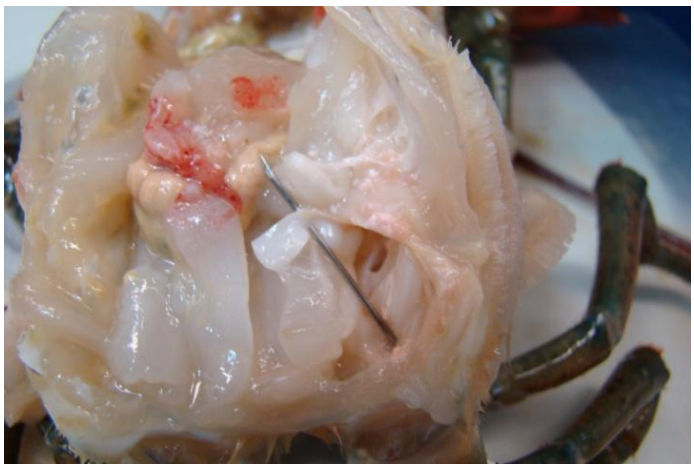

Figure 3- The needle path used for anesthesia of *H. americanus* which delivered the dose to the pericardial sac and reduced damage to surrounding tissues.

Figure 4- The needle path for anesthesia and euthanasia of *C.maenas* which delivered the dose to the pericardial sac. Also seen is gonadal tissue adjacent the tip of the needle.

Figure 5- Injection of euthanizing dose of eugenol mixed with methyl red to visualize the path of injection into *C. maenas*. The dye was concentrated between muscle and the pericardial sac. The gills on the side of injection were dyed signifying that the eugenol solution with the dye had entered the sinusoidal circulatory system.

Figure 6- Injection of euthanizing dose of eugenol dyed red with methyl red into a lobster. The dye was concentrated between muscle and the pericardial sac. The gills on the side of injection were dyed signifying that the eugenol solution with the dye had entered the sinusoidal circulatory system.

Figure 7- Injection site for a euthanizing dose of eugenol in *H. americanus.* The needle was inserted at the arthrodial membrane that creates the joint between the hard carapace of the abdomen and the cephalothorax.

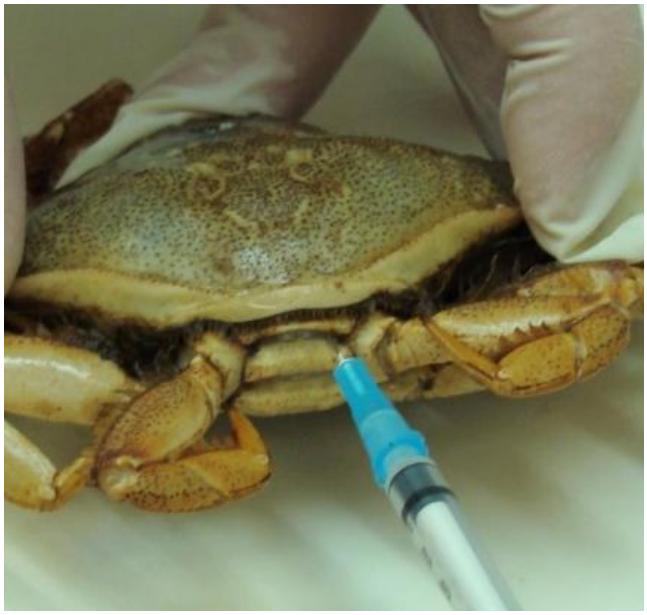

Figure 8- Injection site for euthanasia and anesthesia used for crabs. The needle was inserted through the arthrodial membrane that forms the joint separating the cephalothorax from the abdomen.

Figure 9- Injection sites for anesthetic doses of eugenol in *H. americanus*. The needle was inserted through the arthrodial membrane forming the joint at the base of the third walking leg.

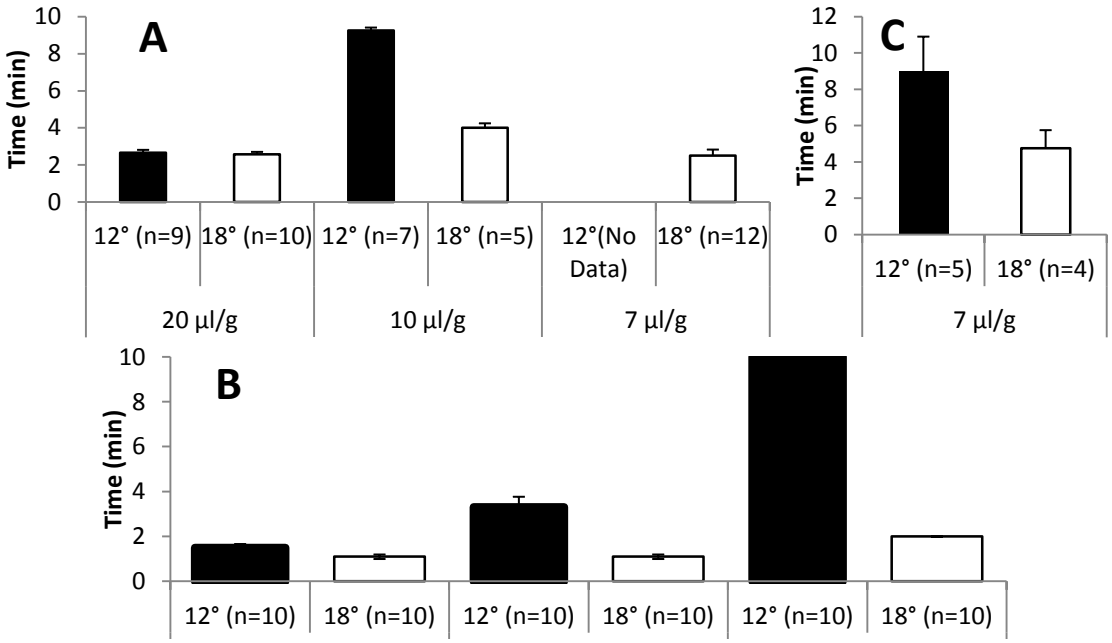

Figure 10 Average time (±SD) until immobile (A3, M2.5, E3) for euthanizing doses of eugenol for (A) *Carcinus meanus* (B) *Cancer irroratus* (C) *Homarus americanus.* Black bars represent animals mantained at 12° White bars are animals mantained at 18°C

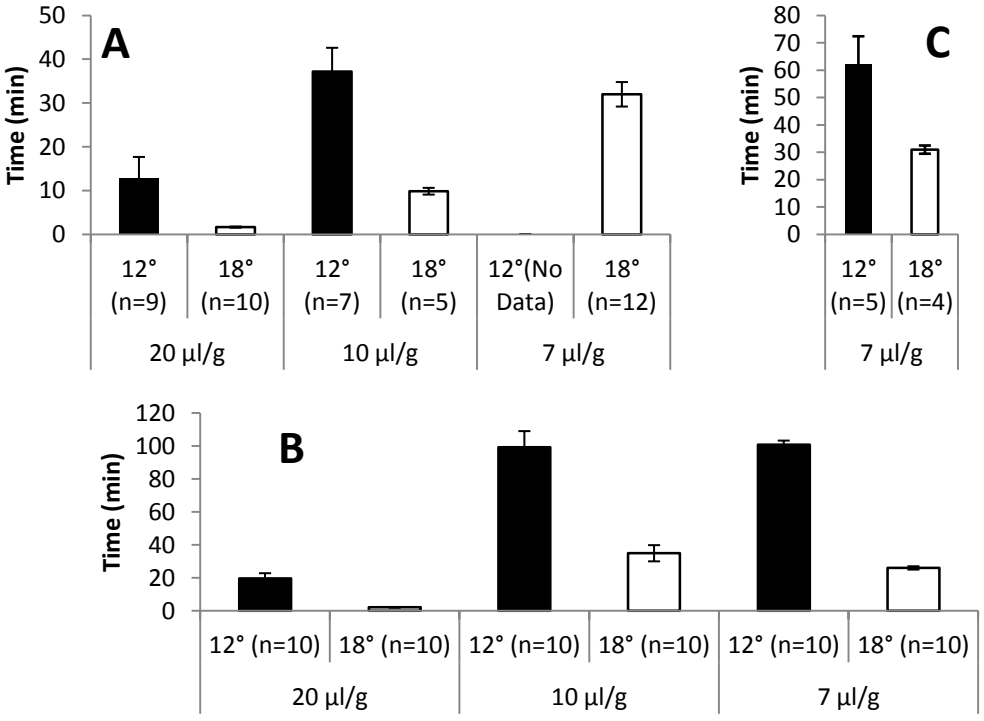

Figure 11 Average time (±SD) until apparent death (A3, M2.5, E3) for euthanizing doses of eugenol for (A) *Carcinus meanus* (B) *Cancer irroratus* (C) *Homarus americanus.* Black bars represent animals mantained at 12° White bars are animals mantained at 18°C

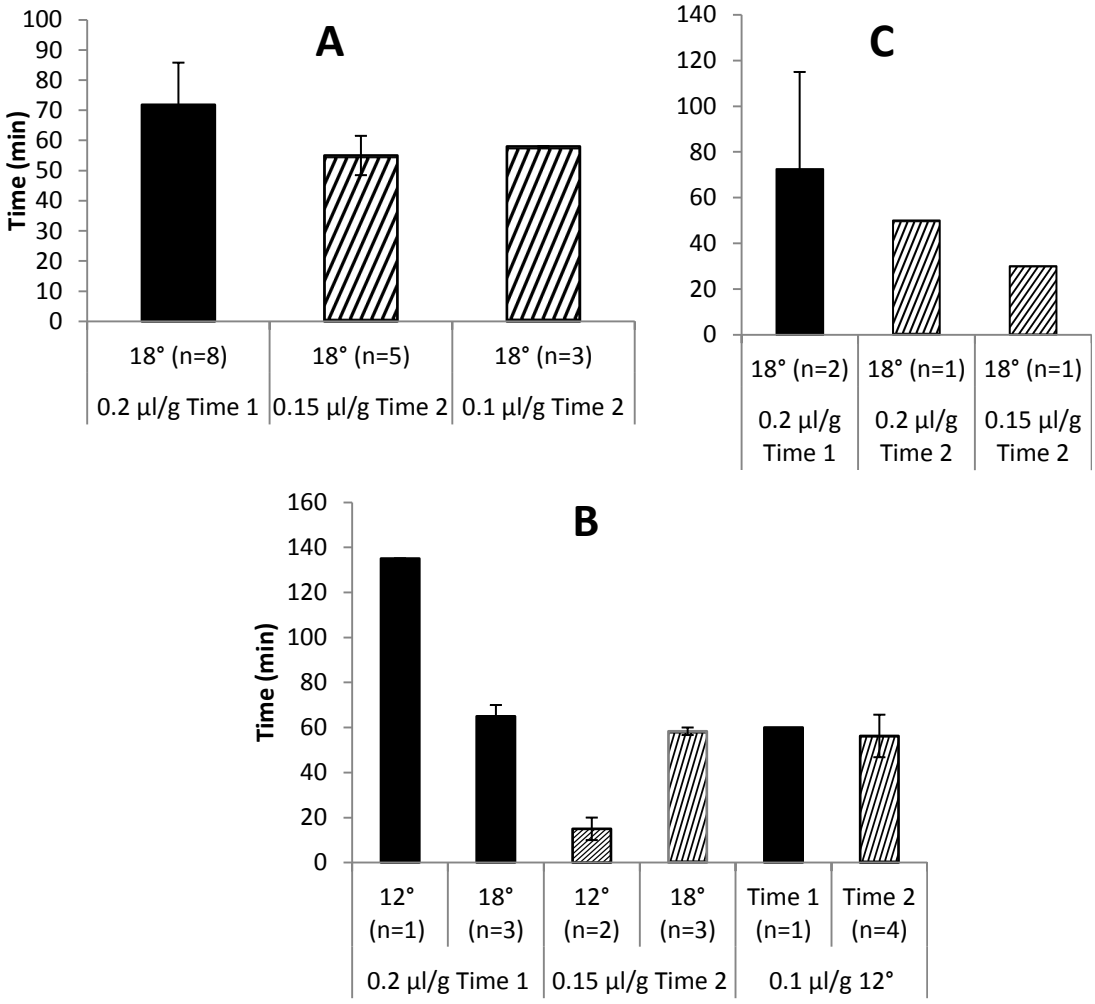

Figure 12-Average time (±SD) anethetized at (A3, M2.5, E3) for anethetic doses of eugenol for (A) *Carcinus meanus* (B) *Homarus americanus* (C) *Cancer irroratus* Black bars represent injected during time 1 (see text) Striped bars are animals injected during time 2

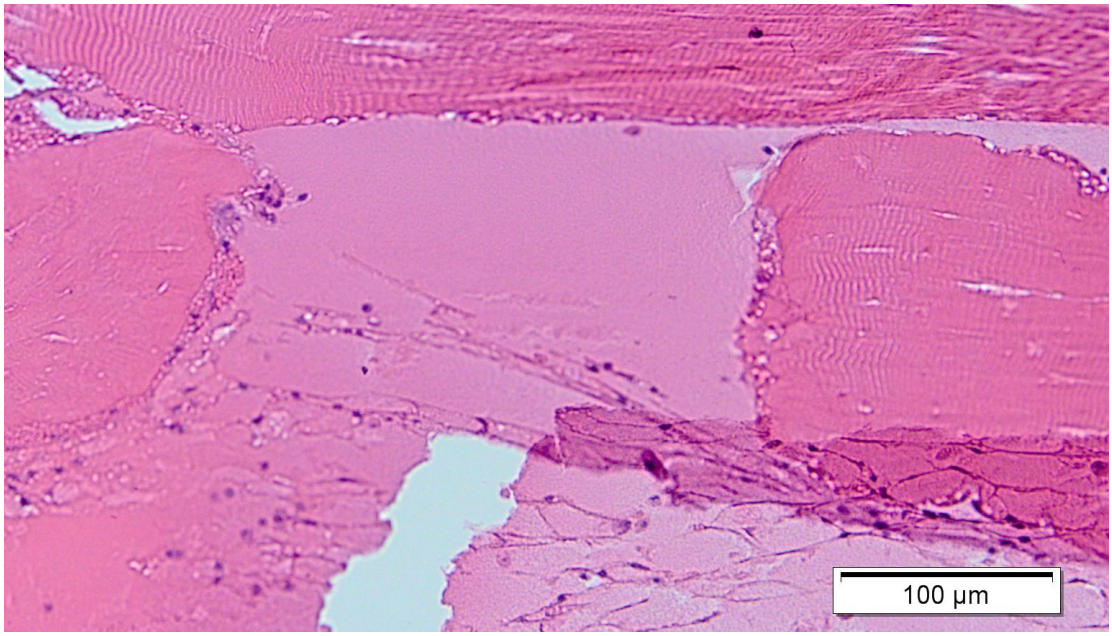

Figure 13-Photomicrograph of a 6 µm section of paraffin embedded tissue stained with hematoxylin and eosin. Peracute necrosis of specific muscle bundles note from tissue sample taken from a green crab removed 3 minutes after an injection of 20 µl/g eugenol. There is significant muscle damage

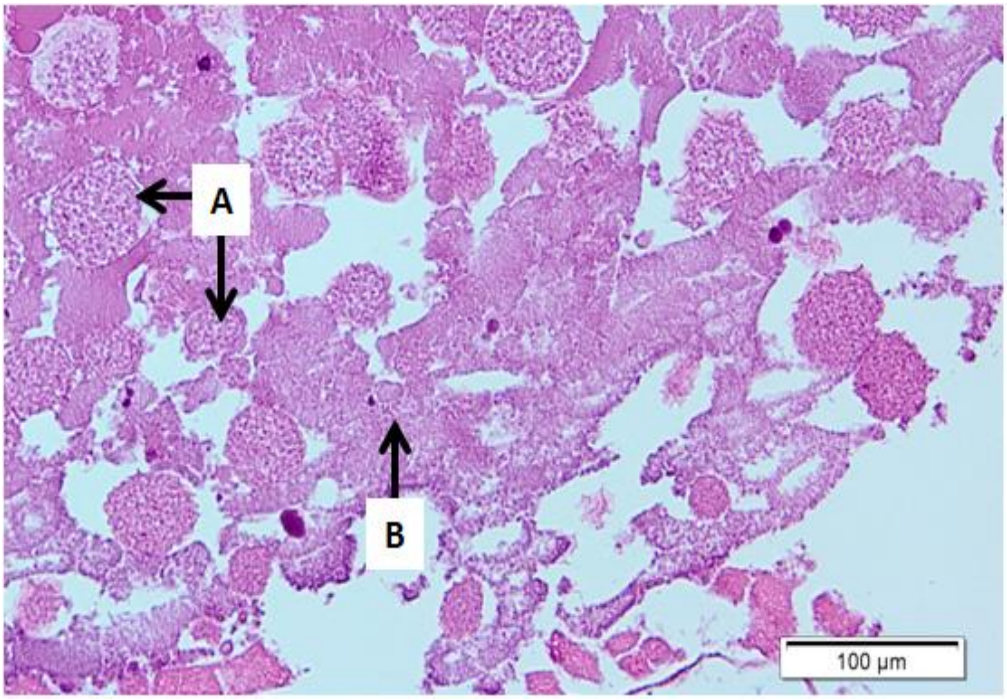

Figure 14-Photomicrograph of a 6 µm section of paraffin embedded tissue stained with hematoxylin and eosin. Noting peracute diffuse sever necrosis of pericadiral muscle intermixed with acute aggregations of hemocytes in the lumen of this tissue removed from a green crab taken 3 minutes after an injection of 20 µl/g eugenol.

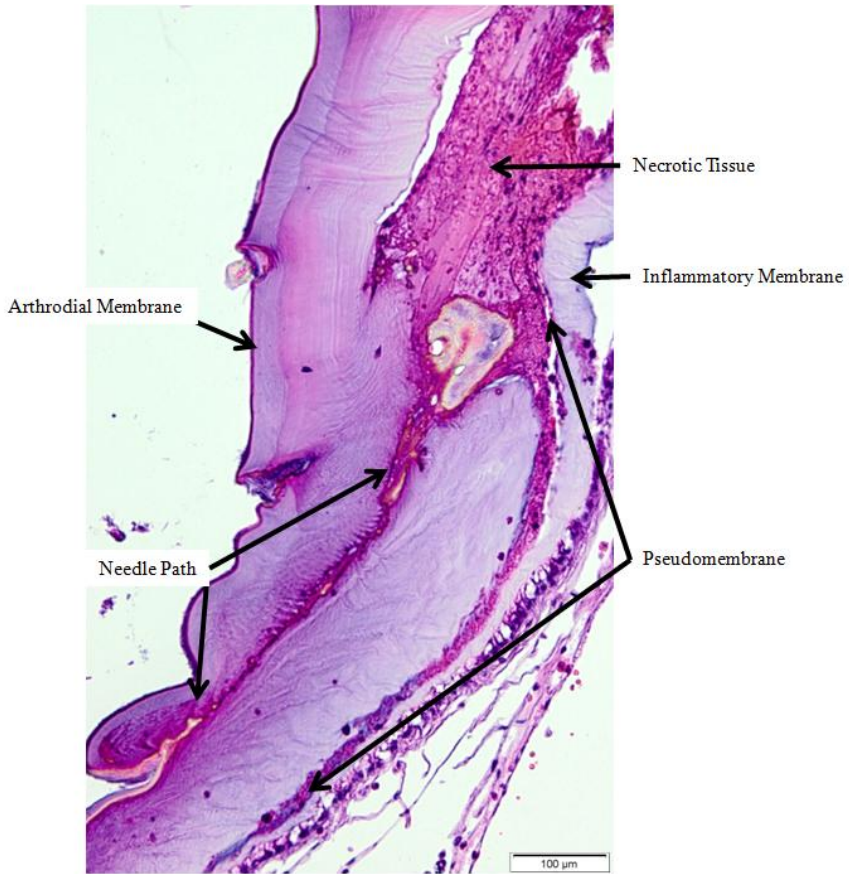

Figure 15- Photomicrograph of a 6 µm section of paraffin embedded tissue stained with hematoxylin and eosin. Note the needle path though the arthrodial membrane at the joint of the third walking leg. The pseudomembrane is separating the newly produced inflammatory membrane from the overlaying necrotic tissue. This tissue section was taken from *H.* americanus at the injection site one month after injection of anesthetic dose (0.15µl/g) of eugenol solution.

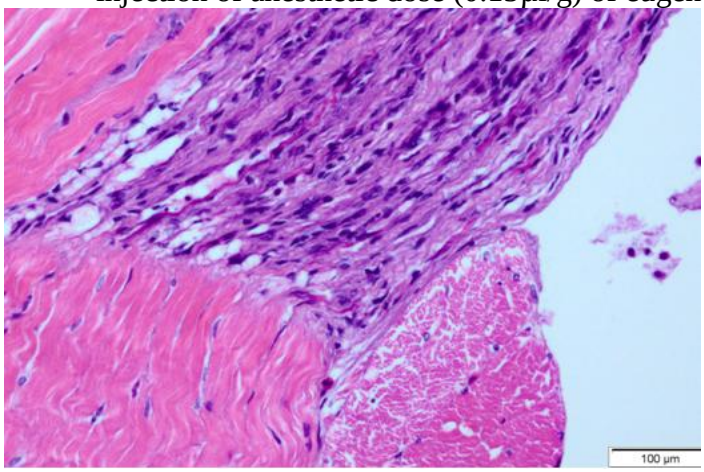

Figure 16- Photomicrograph of a 6 µm section of paraffin embedded tissue stained with hematoxylin and eosin. Muscle sample taken at 30 days post-injection of an anesthetic dose of eugenol (0.15µl/g). Muscle bundle regeneration is present and characterized by newly forming fibers with numerous blue nuclei.

Appendix

List of tables

Table i- Data for all *H. americanus* injected with an euthanizing dose of eugenol. Each row represents a unique lobster

dose (µl/g)	temp °C	season	Mass (g)	Carapace length (mm)	sex	Time (min) until unresponsive (A3 M2.5 E2)	Time until euthanized (min)
7	12	Time 2	444	83.2	Male	5	95
7	12	Time 2	454	82.8	Female	10	78
7	12	Time 2	396	85	Male	10	46
7	12	Time 2	583	86	Male	10	49
7	12	Time 1	565	86	Male	10	42
7	18	Time 2	461	85.5	Male	5	30
7	18	Time 2	361	82.5	Male	2	35
7	18	Time 2	238	64.8	Male	2	32
7	18	Time 2	502	84	Male	10	28

Table ii- Data for all *C. maenas* injected with a euthanizing dose of eugenol. Each row represents a unique animal

dose (µl/g)	temp °C	Season	Mass (g)	Carapace Width (mm)	sex	Time (min) until unresponsive (A3 M2.5 E2)	Time until euthanized (min)
20	18	Time 1	85.3	67.3	Male	1	2.25
20	18	Time 1	115	70.5	Male	1	1.75
20	18	Time 1	125	75.9	Male	1	1.37
20	18	Time 1	123	73.6	Male	1	1.52
20	18	Time 1	87.4	73	Male	1	1.2
20	12	Time 1	71.5	61.3	Male	1	10.5

Table ii Continued

dose (µl/g)	temp °C	Season	Mass (g)	Carapace Width (mm)	sex	Time (min) until unresponsive (A3 M2.5 E2)	Time until euthanized (min)
20	12	Time 1	95.6	66	Male	1	10.76
20	12	Time 1	110	69.7	male	1	50
20	12	Time 1	89.4	65.9	Male	1	18.88
20	12	Time 1	108	71.2	Male	2	5.8
20	18	Time 1	139	81	Male	2	2.3
20	18	Time 1	81.8	70.3	Male	1	1.23
20	18	Time 1	138	76.4	Male	1	1.52
20	18	Time 1	46.3	55	Female	2	2.25
20	18	Time 1	110	72.2	Male	1	1.28
20	12	Time 1	85.6	65.6	Male		no response
20	12	Time 1	95.5	69.9	Male	2	5.3
20	12	Time 1	98.5	73.3	Male	1	2.1
20	12	Time 1	108	71.8	Male	1	6.5
20	12	Time 1	78.9	61.9	Male	1	5.3
10	18	Time 1	74.1	63.2	Female	2	12.25
10	18	Time 1	77.5	61.2	Male	2	8.3
10	18	Time 1	115	75.2	Male	2	10.8
10	18	Time 1	65.8	63.5	Female	3	8.1
10	18	Time 1	55.4	60.3	Female	3	9.9
10	12	Time 2	35.8	49.8	Male	2	15.5
10	12	Time 2	41.7	50	Male	2	32.5
10	12	Time 2	54	55	Female	2	23.5
10	12	Time 1	45.9	52.5	Female	10	49
10	12	Time 1	45.5	53.8	Female	10	55
10	12	Time 1	45.1	51.3	Female	10	47
10	12	Time 1	52.4	56	Male	10	38
7	18	Time 2	41.3	52	Male	2	41
7	18	Time 2	73.5	72	male	2	43
7	18	Time 2	73.5	62	Male	2	33
7	18	Time 1	53.2	55.5	Female	2	32
7	18	Time 1	43.3	56	Female	3	33
7	18	Time 1	42.5	54	Female	2	31
7	18	Time 2	69.3	64	Female	5	13
7	18	Time 2	81	71	Male	5	47
7	18	Time 2	61.3	57	male	2	37
7	18	Time 2	52.3	60	Female	2	17
7	18	Time 2	39.5	52	Female	2	32
7	18	Time 2	93.2	69	Male	2	26

Table iii- Data for all *C. irroratus* injected with a euthanizing dose of eugenol. Each row represents a unique animal

dose (µl/g)	temp °C	Season	Mass (g)	Carapace width (mm)	Sex	Time (min) until unresponsive (A3 M2.5 E2)	Time until euthanized (min)
20	18	Time 1	218	104	Male	1	2.3
20	18	Time 1	173	96.9	male	1	1.97
20	18	Time 1	267	109	Male	1	2.1
20	18	Time 1	202	101	Male	1	1.84
20	18	Time 1	222	109	Male	1	1.88
20	12	Time 1	218	98.2	Male	1	12.25
20	12	Time 1	254	107	Male	1	11.25
20	12	Time 1	267	98.5	Male	2	35.1
20	12	Time 1	202	104	Male	2	32.25
20	12	Time 1	222	106	Male	2	34.07
20	18	Time 1	205	101	Male	1	1.72
20	18	Time 1	213	100	Male	1	1.82
20	18	Time 1	212	104	Male	1	1.3
20	18	Time 1	185	99.7	Male	2	2.3
20	18	Time 1	184	101	Male	1	3.52
20	12	Time 1	205	94.4	Male	2	21.24
20	12	Time 1	213	95	Male	1	9.8
20	12	Time 1	212	108	Male	1	12.5
20	12	Time 1	185	102	Male	1	12.45
20	12	Time 1	184	106	male	2	15.52
10	12	Time 1	188	98.9	Male	5	123
10	12	Time 1	195	97.8	Male	2	106
10	12	Time 1	261	111	Male	2	52.2
10	12	Time 1	270	115	Male	2	131.25
10	12	Time 1	195	97.4	male	2	43.25
10	12	Time 1	259	108	Male	2	96
10	12	Time 1	220	106	male	3	91
10	12	Time 1	209	101	Male	5	100
10	12	Time 1	183	97.5	Male	5	114
10	12	Time 1	223	107	Male	5	135
10	18	Time 1	170	96.2	Male	1	42.3
10	18	Time 1	181	98.1	Male	1	3.52
10	18	Time 1	270	111	Male	1	31
10	18	Time 1	176	95.5	Male	2	32
10	18	Time 1	183	96.4	Male	1	22

Table iii continued

dose (µl/g)	temp °C	Season	Mass (g)	Carapace width (mm)	Sex	Time (min) until unresponsive (A3 M2.5 E2)	Time until euthanized (min)
10	18	Time 1	168	85.8	Male	1	57
10	18	Time 1	284	113	Male	1	37
10	18	Time 1	200	98.4	Male	1	50
10	18	Time 1	201	104	Male	1	27
10	18	Time 1	170	95.3	Male	1	48
7	12	Time 1	219	99.2	Male	10	100
7	12	Time 1	179	96.4	Male	10	95
7	12	Time 1	166	97.5	Male	10	100
7	12	Time 1	275	109	Male	10	115
7	12	Time 1	188	97	Male	10	95
7	12	Time 1	189	103	Male	10	100
7	12	Time 1	112	103	Male	10	100
7	18	Time 2	78.1	76	Male	2	25
7	18	Time 2	55.6	68	Male	2	27

Table iv Data for all *H. americanus* injected with an anesthetic dose of eugenol. Each row represents an unique lobster.

Dose (µl/g)	temp °C	Season	Mass (g)	Sex	Time (min) until A3 M2.5 E2	Time until recovery (min) A1,M1,E1	Recovery Level
0.2	12	Time 1	553	Male	45	180	Full Recovery
0.2	18	Time 1	460	Male	Light	75	Full Recovery
0.2	18	Time 1	575	Male	Light	60	Partial recovery
0.2	18	Time 1	497	Male	Light	60	Full Recovery
0.2	18	Time 1	238	Male			Almost no effect
0.2	18	Time 2	443	Male	15		Died after 1 day
0.15	18	Time 2	502	Male	15	75	Partial recovery
0.15	18	Time 2	290	Male	15	75	Partial recovery
0.15	18	Time 2	178	Male	5	60	Full Recovery
0.15	12	Time 2	389	Female	10	30	Partial recovery
0.15	12	Time 2	533	Male	10	20	Full Recovery
0.1	12	Time 1	565	Male	Light	60	Full Recovery
0.1	12	Time 2	438	Female	10	50	Partial recovery
0.1	12	Time 2	543	Male	10	50	Full Recovery
0.1	12	Time 2	443	Female	15	90	Partial recovery
0.1	12	Time 2	432	Female	20	90	Partial recovery

Table v Data for all *Carcinus meanus* injected with an anesthetic dose of eugenol. Each row represents an unique crab. All crabs were collected by metal traps and directly brought into the lab.

Dose (µl/g)	temp °C	Season	Mass (g)	Sex	Time (min) until A3 M2.5 E2	Time until recovery (min) A1,M1,E1	Recovery Level
0.2	12	Time 1	55.8	Male			No response
0.2	18	Time 1	78.9	Male	10	45	Full Recovery
0.2	18	Time 1	41.9	Female	10	45	Full Recovery
0.2	18	Time 1	58.5	Female	10	45	Partial recovery
0.2	18	Time 1	72	Female		120	Full Recovery
0.2	18	Time 1	54	Female	15	90	Full Recovery
0.2	18	Time 1	80.6	Male	15	60	Full Recovery
0.2	18	Time 1	36	Female	5	120	Full Recovery
0.2	18	Time 1	56.7	Female	5	120	Full Recovery
0.2	18	Time 2	69.3	Male	5		Died after 1 day
0.2	18	Time 2	38.3	Female	5		Died after 1 day
0.2	18	Time 2	84.5	Male	5		Died after 1 day
0.2	18	Time 2	54.8	Female	5		Died after 1 day
0.2	18	Time 2	38.2	Female	5		Died after 1 day
0.2	18	Time 2	94.2	Male	5		Died after 1 hour
0.2	18	Time 2	68.4	Female	5		Died after 1 hour
0.2	18	Time 2	40.2	Female	10		Dead after 2 hours
0.15	18	Time 2	24.8	Female		60	Full Recovery
0.15	18	Time 2	68.3	Female	10	75	Full Recovery
0.15	18	Time 2	51.6	Female	10	75	Full Recovery
0.15	18	Time 2	40.2	Female	20	75	Full Recovery
0.15	18	Time 2	42.7	Female	20	50	Full Recovery
0.1	18	Time 2	41.1	Female	2	60	Full Recovery
0.1	18	Time 2	73.2	Male	5	60	Full Recovery
0.1	18	Time 2	67	Male	2	60	Partial recovery

Table vi- Data for all *Cancer irroratus* injected with an anesthetic dose of eugenol. Each row represents an unique crab. All crabs were collected by metal traps and directly brought into the lab

Dose (µl/g)	temp °C	Season	Mass (g)	Sex	Time (min) until A3 M2.5 E2	Time until recovery (min) A1,M1,E 1	Recovery Level
0.2	18	Time 1	72.2	Male	5	120	Full Recovery
0.2	18	Time 1	82.9	Male	30	60	Full Recovery
0.2	18	Time 2	78.3	Male		30	Full Recovery
0.15	18	Time 2	77.9	Male	10	60	Full Recovery