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EFFICACY OF ANESTHETICS FOR REDUCING STRESS IN FISH DURING AQUACULTURE PRACTICES- A REVIEW

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ABSTRACT
Intensification of aquaculture practices has increased stress level in fish. Transportation, handling, sorting by size, weighing, increased farming density, confinement level, and degradation of water quality are stressors in aquaculture practices which exhibit physiological stress responses in fish. These stressors bring changes in plasma cortisol, glucose, lactase, plasma chloride, sodium, and lymphocyte concentrations in fish. Fish stress and mortality can cause significant losses of resources and productivity in both capture and culture systems. Use of anaesthetics with optimum concentration could mitigate the stress and its related harm in fish, by increasing fish welfare, production, and profitability. This review has summarizes the aquaculture stress, its related consequences to fish, and efficacy of anaesthetics to reduce these factors.

Kew words: Aquaculture, anaesthetics, stress, stressors, fish welfare

INTRODUCTION
Fish demand is increasing due to growing population and awareness about health benefits of aquatic animal food. However, due to decline in capture fisheries worldwide, aquaculture is only option to meet the demand of fish [1]. To meet the ever-increasing demand of fish as an animal protein, globally aquacultural practice becoming more and more intensive. However, with every step towards intensification of aquaculture practices, there is an increase in stress level on the animal as well as on the environment [2, 3]. Aquaculture has been the fastest growing food producing industry worldwide in the past three decades [4]. During aquaculture and stocking activities, fishes are faced with several potential stressors. In particular, transportation, capture and handling procedures, a highly crowded and confined farming environment, possible air exposure and variation in water quality are all factors that may increase the stress level of organisms [5, 6, 7, 8] and have significant effects on fish physiology and survival[9,10]. Stress is often associated with disease outbreaks in cultured fish [11,12]. Fish stress and mortality can cause significant losses of resources and productivity in both capture and culture systems [13, 14]. Reduction of these losses requires knowledge of stressors, stress levels and fitness outcomes [14, 10]. Fish welfare is compromised by stress and has become an increasing concern in the operation of capture, rearing and research operations [16, 17, 18, 19, 20]. They emphasized on the impact of various aspects of aquaculture activities (transportation, handling and netting, confinement and short-term crowding, inappropriate densities, water quality deterioration) effects on fish welfare and the resulting harm to fish welfare must be minimized and weighed against the benefits of the activity concerned [17].

Stress is a condition in which animal organisms homeostasis is threatened or disturbed as a result from actions of stressors [10]. The stress response in fish is separated into two concepts defined as 'adaptive' or 'maladaptive'. Acute stress commonly caused by handling or abrupt environmental changes, are designated as adaptive as the fish most likely will be able to recover. When subjected to high density and bad water quality, stress response might become maladaptive as the fish are unable to escape. If the stress load gets chronic, the metabolic energy will be reallocated from the investment activity toward activity for restoring homeostasis [5]. The increased metabolic cost for coping with the stress will eventually cost the fish its health and well-being. Overall the stress load will affect its physiological system, causing reduced growth, inhibit reproduction and suppress its immune function. Eventually the fish will be exhausted and is likely to incur disease and die [5, 10, 14].

Physiological responses of fish to environmental stressors are grouped broadly as primary and secondary.The primary responses are the perception of an altered state by central nervous system and the release of stress hormones, cortisol and catecholamine into the blood circulation by endocrine system [5, 10, 21]. Primary stress responses trigger the sequential secondary response (e.g. increase in plasma glucose, lactate and hematocrit and decrease in chloride, sodium and potassium) in teleosts [5]. Additionally, tertiary responses occur, which refer to aspects of whole-animal performance such as changes in growth, condition, overall resistance to disease, metabolic scope for activity, behaviour, and ultimately survival [5, 10]. Capture, handling, crowding, confinement and transport are all components of aquaculture, which influence the physiological stress response in fish [22, 23, 24]. Cortisol and glucose are two of the most common stress indicators in fish [21].

In aquaculture, sedative and anesthetic agents are very useful for reducing the stress caused by handling, sorting, transportation, artificial reproduction, tagging, administration of vaccines and surgical procedures [25, 26]. They are also used to immobilize fish so they can be handled more easily by biologists during blood sampling and research experiments. Anaesthetics in fish farms are used to minimize mortality during handling and transport. This may reduce susceptibility to pathogens and infection. Anaesthetics are also used in fish during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical procedures [27]. Knowledge about the ideal and optimum concentration of an anaesthetic for various fish species is necessary because inappropriate concentrations may lead to adverse effects such as stress [28, 29, 30]. Therefore, access to safe and effective fish sedatives is a critical need of fisheries researchers, managers, and culturists [31].
The aim of this paper is to present stressors and stress in fish, stress responses by fish, anesthetics and its efficacy to reduce stress, and to give an overview of recent literature reports related to efficacy of anaesthetics.

MATERIALS AND METHODS

Scientific literatures were searched from journal articles, books, proceedings and others published article starting from the year 2000 to the year 2013. In the light of these scientific literatures, this review paper related to efficacy of anaesthetics for reducing stress in aquaculture practices was prepared. The major journals papers included in this review paper were from the journals: Aquaculture; General and Comparative Endocrinology; Comparative Biochemistry and Physiology Part A; Aquaculture Research; Fish physiology and Biochemistry; Integrative and Comparative Biology; Journal of Fish Biology. The others journals papers were from the journals: Acta veterinaria Brno; Acta Amazonica; Journal of the World Aquaculture Society; Journal of Applied Aquaculture;Veterinarni Medicina;The Israeli Journal of Aquaculture – Bamidgeh; Transactions of the American Fisheries Society; General and Comparative Endocrinology; North American Journal of Aquaculture; Iranian Journal of Fisheries Sciences; Czech J. Anim. Sci.; Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science; Zoologia ; Journal of Applied Ichthyology; Journal of Animal Physiology and Animal Nutrition; The Bangladesh Veterinarian; Pan-American Journal of Aquatic Sciences; Archives of Polish Fisheries; Ciência Rural; Journal of Aquatic Animal Health; Bulgarian Journal of Agricultural Science; Natura Rerum; Diseases of Aquatic Organisms. Others related scientific papers and articles were also included.

RESULTS AND DISCUSSION

1. Aquaculture practices and stress in fish

Modern aquaculture practices frequently expose fish to a variety of acute stressors that have the potential to negatively affect fish performance and survival. During mass production of fish, the most important causes of stress are those related to various operations: transportation, sorting by size, weighing etc, increased farming density, confinement level, and degradation of water quality [8, 32, 33]. Fish transport is one of the most stressful procedures in aquaculture facilities [34]. Fish are easily stressed by handling and transport, which can result in immuno-suppression, physical injury, or even death [35]. common practices of fish farming, such as capture, confinement, transport and water quality are stressful to fish and can therefore increase the incidence of disease and mortality and impair growth and survival [13].Transportation and handling procedures consists of several potential stressors, such as capture, on-loading, transport, unloading, temperature differences, water quality changes and stocking [6, 10, 36, 37, 38, 39]. Short term crowding stress occurs commonly in aquaculture practices; possess characteristics of acute as well as chronic stress with long-term compromised immune systems resulting in disease or death [10]. Temperature, dissolved oxygen, ammonia, nitrite, nitrate, salinity, pH, carbon dioxide, alkalinity and hardness in relation to aluminium and iron species are the most common water quality parameters affecting physiological stress [6, 10, 40, 41, 42].

2. Stressors and stress response by fish

Routine aquaculture procedures such as, handling, netting, crowding, confinement and live transport evoke stress response in fish [43]. Pre-transport fish manipulation (hauling, netting, and handling, loading) are found to be an important stressor for fish [44]. Transport for short or long durations can be stressful to fish [45]. It was found that transport stress response on the primary (cortisol) and secondary (glucose) levels in Brook charr (*Salvelinus fontinalis*) [46]. It was demonstrated that transportation stress causes reduction in immunity of catla (*Catla catla*) seed [43]. The authors found significant changes in glucose, lactate, MCH and MCHC in Black goby (*Gobius niger*) due to capture and handling [47]. They found that transport of juvenile winter flounder (*Seudopleuronectes mericanus)* produced elevated cortisol levels as a stress response at all stocking densities [48]. There was a significant stepwise increase in corticosterone levels in stingrays over transport time in combination with osmoregulatory disturbances suggesting a stress related role of this corticosteroid in *Potamotrygon cf. histrix* (Cururu stingray) fish [49]. Transportation may be considered a strong stressor to catfish [50]. Due to the effect of 12 h transport to three-years-old common carp, the levels of glucose, lactate, alanine aminotransferase and calcium were significantly changed which indicated that pre-transport manipulation and transport *per se* were found to be important stressors [44]. Stress associated with capture from the wild, handling and transport and shallow water evoked significant changes in circulating levels of cortisol, glucose, lactate, haemoglobin (Hb) and haematocrit (Hct) in coral trout (*Plectropomus leopardus)* [51]. Some haematological indicators (Hb, MCH and MCHC) significantly influenced by the stocking density of the European catfish, *Silurus glanis* [52].

Exercise and exposure to air create stress with significant increased in lactate production, hyperglycemia, plasma Ca^{2+}, Cl^{-} and Na^{+} in bonefish (*Albula vulpes*; a tropical marine fish) [53]. Sturgeon (*Acipenser fulvescens)* stressed by a brief aerial exposure in groups or isolation [54]. Enforced exercise induced a moderate stress response in juvenile turbot (*Scophthalmus maximus*, Rafinesque) reared at various temperature and salinity combinations [55]. The effects of confinement and exercise on the stress response of the spiny damselfish, *Acanthochromis polyacanthus* showed that plasma cortisol concentrations increased in response to stress with a latency period of 5–10 min [56]. Swimming exercise increased in concentrations of plasma lactate and glucose and lower water temperature acted as an environmental stressor to Pacific cod (*Gadus macrocephalus tilesius*) [57].

They found that long-term handling stress (15 s out of water, each day, for 4 weeks) in haddock (*Melanogrammus aeglefinus*), free and total plasma cortisol levels increased significantly (10-fold) in the stressed group[58]. The results demonstrated that pirarucu (*Arapaima gigas*) exhibit physiological stress responses to handling [59]. There is a possible link in *S. salar* mucus molecular indices and the response to handling stress as indicated by a shift towards increased actin fragmentation over time and correlation between lysozyme and cortisol [60]. It was confirmed that cortisol concentrations in water and estimated cortisol release rates increased in response to handling stress, and that both were correlated with plasma cortisol concentrations in rainbow trout (*Oncorhynchus mykiss*) [61].

Plasma glucose and free fatty acid levels were elevated after 30 mins and 3 hour net confinement [62]. Six hour period of confinement resulted in changes to plasma cortisol, glucose, amino acid and lactate levels compared with unconfined controls in six species of freshwater fishes, from the families Cyprinidae; common carp (*Cyprinus carpio*), roach (*Rutilus rutilus*) and chub (*Leuciscus cephalus*) and Salmonidae; rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) [63]. It was found that in response to 3 h net confinement stress, the plasma cortisol level reached a peak at one hour post-stress (85-fold higher than in control) in Common carp (Cyprinus carpio L.) [64]. Confinement as a stressor increased plasma glucose and lactate levels in Rainbow trout (*Oncorhynchus mykiss*) [65]. They found that when Sunshine bass (*Morone chrysops* and *Morone saxatilis*) were subjected to a 15-min low-water confinement stressor, an initial increase in hematocrit, followed by a delayed decrease in hematocrit and chloride, and an increase in plasma glucose and cortisol [23] . Decrease in plasma chloride, sodium and lymphocyte and increase in the values of glucose and neutrophils were the main changes observed in kutum (*Rutilus frisii* kutum) following captivity which may indicative of the responsiveness of brood stocks to stress [66].

Plasma cortisol levels increased significantly of both the species of two Indian major carps, rohu (*Labeo rohita*) and mrigala (*Cirrhinus mrigala*) with increasing temperatures [67]. They found that the Grouper (*Epinephelus Malabaricus*) fish exhibited different responses to ambient salinity stress in relation to salinity changes [68]. Electro fishing induces a general stress response in Chub (*Leuciscus cephalus*) as it exhibited rapid elevations in plasma glucose and blood lactate levels [69].

3. Efficacy of anesthetics in reducing stress in fish

One of the methods to mitigate stress in fish is by use of anesthetics in various aquaculture activities [70, 71]. Anaesthetics are used in aquaculture and fisheries to facilitate various routine procedures including: capture, handling, transportation, tagging, grading and measurements that can often cause injury or induce physiological stress in aquatic species [72]. Anesthetics have been evaluated for their ability to elicit or inhibit a corticosteroid response apart from that which may be a result of handling or exposure to an acute stressor (36, 73). Anesthetics are used to immobilize fish so they can be handled more easily during harvesting, sampling and spawning procedures [35]. To immobilize fish, reduce stress levels, and prevent mortality by use of anesthetics have been practiced by fish handlers [74]. Sedative and anaesthetic agents are very useful in aquaculture as they reduce fish activity, limit oxygen consumption, and facilitate such routine handling operations as weighing, measuring, sorting, manual spawning, marking, and veterinary procedures. Their main advantage is to minimize stress reactions during high density storage and transport [75]. Anaesthetics are used in aquaculture and fisheries to facilitate routine procedures: capture, handling, transportation, tagging, grading and measurements that can often cause injury or induce physiological stress [76]. Anaesthetic agents added to the water at low doses may also be used to sedate fish prior to transport. This reduces metabolic rate and hence oxygen demand, reduce general activity, increase ease of handling, and mitigate the stress response [77].When choosing an anaesthetic, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment (78, 72, 79]. Anesthesia is essential to minimize stress and physical damage during handling of fish in captivity [80]

Widely used drugs for inhalation anaesthesia in fish are; MS222 Clove oil, Benzocaine, Aqui-s, 2 Phenoxy ethanol and Metomodiate [35, 72, 81]. The efficacy of these above anaesthetics is described here.

Clove oil

Clove oil is a natural product obtained by distillation of the flowers, stems and leaves of the clove tree *Syzygium aromaticum* (i.e. *Eugenia aromaticum* or *Eugenia caryophyllata)*. It is a dark brown liquid with a rich, aromatic odour and flavour. It has been used as a mild topical anaesthetic since antiquity and to help with toothache, headaches and joint pains. Clove is relatively inexpensive [35, 72, 81]. It can be recommended as a very suitable, economically advantageous and easy accessible anaesthetic for fish. It refers to natural substance which has no any side-effects in fish and does not represent any ecological neither hygienic risks [82]. It has high efficacy in small doses, non-specific toxicity and in particular cost-efficiency, has been major reasons for its adoption in aquaculture and aquatic research [36, 78]. It has been proposed that clove oil is a reasonable alternative as fish anaesthetic [83, 84].

Clove oil has suppressive effects on plasma cortisol and reduces the plasma lactate response [73, 85]. Its concentration about 40 mg L^{-1} is enough to anaesthetize the fish in approximately one minute[86] and at dose (5 mg L^{-1}) can mitigate the stress response in matrinxã (*Brycon Cephalus*) subjected to transport [34]. Low level of clove oil (5 to 9 mg L^{-1}) is effective for mitigating the effects of fish transport stress in sub adult largemouth bass [77]. Clove oil can be used as an effective and efficient agent for anesthesia in the aquaculture of European sea bass and gilthead sea bream [25]. A concentration of 15 mg L^{-1} clove oil could safely be used to transport post larvae of the freshwater prawn, *Macrobrachium rosenbergii* up to three hours [87]. Eugenol-based anaesthetics (Clove oil) appear to be promising as a stress-reducing sedative for Atlantic salmon *Salmo salar L* smolts, and, if used properly, this chemical could improve animal welfare and survivability during and after common aquaculture-related incidents [88]. It was found to be the most effective in reducing the short-term stress induced by routine biometry (20 mg L^{-1},10 min) and also by transporting (1mg L^{-1}, 8 h) for juvenile cobia (*Rachycentron canadum*) [89]. The results indicated that eugenol (clove oil) can be used as a sedative during transportation of post larvae of Indian shrimp *F. indicus* and concentration of 1.3 mg L^{-1} is considered safe [90]. Clove oil identified as most effective and an ideal fish anaesthetic to seahorse *Hippocampus kuda* (Bleeker, 1852) husbandry in terms of stress, survival and production efficiency [91]. Eugenol is recommended for use in the transport of the silver catfish (*Rhamdia quelen*) species because this anesthetic apparently reduces stress during the transport [92]. Clove oil at the dose of 2 ml L^{-1} is recommended for fresh water angelfish (*Pterophyllum scalare*) for aquaculture activities, such as handling, catching with net, transporting to another tank [93]. Anaesthetic concentrations of clove oil suitable for handling and transport found to be 5 mg $L^{-1}$for uses on marbled spinefoot (S. *Rivulatus*) [76].

Clove oil is more effective at reducing the short-term stress response induced by handling and blood sampling, and is recommended as an alternative fish anaesthetic for rainbow trout (*Oncorhynchus mykiss*) [94]. They concluded that eugenol(clove oil) appears to be a safe anesthetic for handling to use for Nile tilapia (*Oreochromis niloticus*, Linnaeus) [95].The use of clove oil during handling for the kelp grouper (*Epinephelus bruneus)* was found safe [96]. Eugenol based anaesthetic formulation is safe and effective for handling of adult *M. rosenbergii* and useful for mitigating handling stress [97]. Clove oil is an efficient anaesthetic for routine fish farming handling procedures for the Nile tilapia (*Oreochromis niloticus)* [98]. Clove oil showed promise as effective anaesthetics for Atlantic salmon (*Salmo salar* L.) based on good efficacy at low dosage and stress-reducing capabilities [36]. Eugenol is an efficient and safe anaesthetic for tambaqui, *Colossoma macropomum (Cuvier)* at the the ideal dose of 65 mg L^{-1} for both juveniles and sub-adults [99]. Clove oil is acceptable for use in culture of Senegalese sole (*Solea senegalensis Kaup 1*858) [26].

They found that clove oil is effective as anaesthetic, for handling purpose at a dose of 20 mg L^{-1} is suitable in 2–5 g while, 40 mg L^{-1} is suitable for 20 g for iridescent shark, *Pangasius hypophthalmus* (Sauvage, 1878) [100]. Clove oil appears to be an effective and relatively safe anaesthetic for salmonids [101]. A concentration of 200 mg L^{-1}clove oil showed rapid anaesthetic and recovery times in the common octopus (*Octopus minor*, Sasaki), indicating its suitability for this species [102]. Clove is a potent anaesthetic for carp, the safest and most effective at the concentrations of 30-50 mg L^{-1} [75]. It is a potential anaesthetic for rainbow trout *Oncorhynchus mykiss* [103, 84] and common carp, *Cyprinus carpio* [75].

They found that the use of clove oil at a concentration of 30 mg L^{-1} does not cause irreversible damage in European catfish (*Silurus glanis* L.) [104].100 mg L^{-1} clove oil is a safe concentration for anaesthesia of the channel catfish (*Ictalurus punctatus)* [85]. It was found that the use of clove oil at a concentration of 30 mg L^{-1} does not cause irreversible damage in rainbow trout [84]. A study demonstrated that clove oil can be used as an effective anaesthetic in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus* *aurata*) aquaculture. The optimal doses of clove oil for the European sea bass (*Dicentrarchus labrax*) found to be around 30 mg L^{-1} and for gilthead sea bream (*Sparus aurata*), 55 mg L^{-1} [25]. They found that clove oil 15.0 mg L^{-1} is effective for sedation for routine weighing and measuring procedures on juveniles and 30.0 mg L^{-1} was effective for performing an ovarian biopsy or tag implantation on adults for black sea bass(*Centropristis striata)* [105]. It was demonstrated that clove oil can be used as an effective anaesthetic measuring length and weight of juvenile Russian sturgeon (*Acipenser gueldenstaedtii*) [106].

Clove oil at concentrations from 0.25 to 0.50 ml L^{-1} acted as effective anaesthetic against four freshwater hardy fishes, *viz.*, *Anabus testudineus, Mystus vittatus, Channa punctatus* and *Channa orientalis* during handling, transportation and experimental purposes [107]. It was confirmed that there is good possibilities of using clove oil as an anaesthetic when carrying out different manipulations on the pike (*Esox lucius* L.) and a concentration of 0.02 ml L^{-1} has a sedative effect [108]. The optimal concentration of clove oil found to be 356 mg L^{-1} to immobilize Siberian sturgeon (*Acipenser baerii* Brandt, 1869) fry [109]. Clove oil concentration of 0.033 ml L^{-1} was sufficient to produce anaesthesia in a majority of fish species; grass carp(*Ctenophyrongon idellus*), black carp (*Mylopharyngodon piceus*), grayling *(Thymallus thymallus),* tench *Tinca tinca*, burbot *Lota lota*, , barbel *Barbus barbus*, rudd *Scardinius erythropthalmus*, trout, pike *Esox lucius* and tilapia(*oreochromis niloticus*), perch *(Perca fluviatilis)* and sterlet *(Acipenser ruthenus*),brook trout *(Salvelinus fontinalis)* and for siberian sturgeon *(Acipenser baeri)* 0.07 ml L^{-1} [82].

The findings suggested that clove oil could be an effective anesthetic for use (doses of 60–100 ppm) for zebrafish, *Danio rerio* (Hamilton), and when compared to MS-222, its benefits include a lower cost, lower required dosage, improved safety, and potentially lower mortality rates[110] . It has clearly revealed that the appropriate safe dose of clove oil as an anesthetic for handling *P. denisonii* is 30 mg L^{-1} [111]. For juveniles of matrinxã (*Brycon cephalus*), the safe clove oil concentrations is ranging from 40 to 50 mg L^{-1} [112]. They found that clove oil is a safer anaesthetic for Gilthead sea bream *(Sparus auratus*) as it does not cause immune depression in anesthetized fish [113]. Clove oil found to be an effective anaesthetic for juveniles of blackspot sea-bream (*Pagellus bogaraveo*) and greater amberjack (*Seriola dumerilii)* at concentrations of 40 and 100 mg L^{-1} respectively [114].

MS222 (Tricaine methane sulphonate)

MS222 compound was first developed by Sandoz. Its base molecule is ethyl 3-aminobenzoate, and it is a white, crystalline powder. It has a good safety margin to fish. It is not currently known to be toxic to humans [30, 35, 72, 81]. MS-222 appears to be highly effective as an anaesthetic with no side effects to both fish as well as humans [80].

MS222 150 mg L^{-1} is required for successful anaesthesia for juvenile *Brycon cephalus* [115]. It was found that optimal MS222 concentration for 20-day-old fingerlings *Perca fluviatilis* during handling (sorting) is 50–70 mg L^{-1}[116]. They found that MS222 for black sea bass *Centropristis striata* doses of 70 mg L^{-1} was effective for sedation for routine weighing and measuring procedures on juveniles and 125 mg L^{-1} was effective for performing an ovarian biopsy or tag implantation on adults [105]. MS-222 concentrations suitable for handling and transport ranged to 10–15 mg L^{-1}, is acceptable for use on marbled spinefoot (*S. Rivulatus*) [76]. It is identified as most effective and an ideal fish anaesthetic to seahorse *Hippocampus kuda* husbandry in terms of stress, survival and production efficiency [91]. It is considered acceptable for use in culture of Senegalese sole (*Solea senegalensis*

*Kaup 1*858) [26]. MS-222 concentration of approximately 90 mg L^{-1} is probably optimal under most circumstances for anesthetization of channel catfish (*Ictalurus punctatus,* Rafinesque) to promote ease of handling while reducing stress [117]. In reducing reflex reactions, MS222 found to be the most effective agents for Atlantic halibut (*Hippoglossus hippoglossus*) [118]. 40 mg L^{-1} dose MS222 is found to be safe during long-term exposure for juvenile cobia (*Rachycentron canadum*) [89]. It was found that MS-222 could be used as sedatives to alleviate transport-related stress in tiger barb (*P. Filamentosus)* to improve their post-transport survival and hence reduce economic loss [119]. Anaesthetization (100 mg L^{-1}) of Arctic charr *Salvelinus alpinus* with MS222 is safe [120]. The effective dose of MS-222 for *Puntius denisonii* is 150 mg L^{-1}. This dosage induced the fish through all stages of anaesthesia, without any mortality [80]. MS-222 was found effective in sedating juvenile cobias *Rachycentron canadum* for the purposes of basic handling and morphometric measurement [121].The results indicated that MS-222 is effective as anaesthetics for juveniles of blackspot sea-bream *Pagellus bogaraveo* and greater amberjack *Seriola dumerilii* at concentrations of 40 and 100 mg L^{-1}, respectively [114].

Benzocaine

Benzocaine is chemically very similar to MS222. It is a white, odourless, tasteless crystalline ester of *p*-amino benzoic acid and ethanol, and as it does not have the MS222 sulphonyl side-group, it is almost totally insoluble in water (only 0.04% w/v), and must first be dissolved in either acetone or ethanol. It is effective at approximately the same doses as MS222. It is not toxic to humans at the concentrations used [35, 72, 81]. It is also less expensive than other available anesthetic compounds [122].

60 mg L^{-1} benzocaine is effective dose for anaesthesation for *Brycon cephalus* [86]. Benzocaine is an effective anesthetic agent for tambaqui juveniles, providing rapid immobilization and rapid recovery, and at the dose of 60 mg L^{-1} was found to be effective for periods of up to 20 min of anesthesia [122]. Its concentrations suitable for handling and transport ranges from 5–10 mg L^{-1} are acceptable for use on marbled spinefoot (*S. Rivulatus*) [76]. Low-dose benzocaine during transport has positive effect on the survival and health of rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) fingerlings [123]. The optimum dose of benzocaine for sedation of juvenile *M. estor* was found to be 15 and 18 mg L^{-1} [124]. Benzocaine is the most effective agents for anaesthesia in reducing reflex reactions in Atlantic halibut (*Hippoglossus hippoglossus*) [118]. It could be used as sedatives to alleviate transport-related stress in tiger barb (*P. Filamentosus)* to improve their post-transport survival and hence reduce economic loss [119]. It was found effective in sedating juvenile cobias (*Rachycentron canadum*) for the purposes of basic handling and morphometric measurement [121]. The optimal concentration of benzocaine is 37 mg L^{-1} for sedation for Siberian sturgeon (*Acipenser baerii* Brandt, 1869) fry [106]. It was concluded that 100 ppm benzocaine, is an effective concentration for the induction of anaesthesia in Crucian carp *Carassius carassius* [125].

AQUI-S

The useful features of clove oil prompted the development of a new anaesthetic compound or fish, named AQUI-S^{R} , at the seafood research laboratory in New Zealand. The active ingredient of the product is isoeugenol, which, although very similar to eugenol, is not present in natural clove oil. AQUI-S is a clear viscous yellow liquid, which is dispersible in freshwater or seawater. It has a gentle action and fish do not usually show any adverse reaction to its presence. It has a wide safety margin at low concentrations. It has been approved for use in New Zealand, Australia and Chile [35, 72, 81, 126].

AQUI-S showed promise as effective anaesthetics for Atlantic salmon (*Salmo salar* L.) based on good efficacy at low dosage and stress-reducing capabilities. It is also easily and inexpensively obtained, and is organic substances safe for the environment and user [36]. AQUI-S vet shows promise as a stress-reducing anaesthetics for European eel (*Anguilla anguilla L.*), and if used properly could improve animal welfare and survivability during and after common ecology- related procedures as capture, tagging and size measuring [127]. 10 mg L^{-1} AQUI-S has enabled diagnostic imaging in *Cyprinus carpio* [128]. AQUI-S was found equally as effective as MS2222 in channel catfish (*Ictalurus punctatus*), and showed some stress-reducing properties [129]. It is suitable for brood fish anesthesia in rainbow trout [130]. Effective doses of AQUI-S found for sedation are 120 ppm for Chinook salmon (*Oncorhynchus tschawytscha)*, and 120 ppm for snapper (*Pagrus auratus)* [131]. It shows promise as a stress-reducing sedative for Atlantic salmon *Salmo salar* L smolts and if used properly could improve animal welfare and survivability during and after common aquaculture-related incidents [132]. It was concluded that an AQUI-S dose of 35 mg L^{-1} is adequate to anesthetize market-sized striped bass *Morone saxatilis* [133]. AQUI-S (eugnol) was found effective in sedating juvenile cobias (*Rachycentron canadum*) for the purposes of basic handling and morphometric measurement [121].

2-Phenoxy ethanol

This is a clear, colourless or straw-coloured oily liquid with a slight aromatic odour which fairly easily passes into solution if shaken with a small quantity of water. The solution has been used as a topical anaesthetic and is bactericidal and fungicidal and because of this additional feature it is useful during laparotomy or abdominal surgery. It has no great advantages over other drugs, but is relatively inexpensive [35, 72, 81, 126]. 2-phenoxyethanol (2-PE) is the most commonly used anesthetic in aquaculture [134]. 2-PE or ethylene glycol monophenyl ether is used for anesthesia in aquaculture, because of its fast effect and quick recovery time. Moreover, it's easy preparation and its low cost makes this chemical very suitable for aquacultural practices [25, 26]. 2-phenoxyethanol appeared to be more suitable for use on marbled spinefoot (*S. Rivulatus*) [76]. The effective anaesthetic concentrations of 2-PE in a number of species of fish have been observed and range is from 0.2 to 0.6 ml L^{-1} [25, 135]. They concluded that 2-phenoxyethanol is promising agents in effectively maintaining a high water quality during the transportation of Indian major carp fry [136]. 2-phenoxiethanol is most effective for use in culture of Senegalese sole (Solea senegalensis Kaup 1858) [26]. It is (0.7 and 0.9 ml L^{-1}) recommended for great sturgeon (*Huso huso*) as eligible doses for haematological studies in this species [137].The results confirmed that the use of 2-phenoxyethanol at a concentration of 0.30 ml L^{-1} does not cause any irreversible damage in sheatfish (*Silurus glanis* L.) [138].The recommended anaesthetic concentration of 0.30 ml L^{-1} 2-phenoxyethanol can be considered safe for rainbow trout (*Oncorhynchus mykiss*) [139]. 2-phenoxyethanol is safe for anaesthetization of Arctic charr *Salvelinus alpinus* [120]. It is a safe anaesthetic for juvenile matrinxã even in exposures up to 600 mg L^{-1} being recommended for many field procedures of fish handling [140].They found that 2-phenoxyethanol, 200.0 mg L^{-1} is effective for sedation for routine weighing and measuring procedures on juveniles and 300.0 mg L^{-1} is effective for performing an ovarian biopsy or tag implantation on adults of black sea bass (*Centropristis striata*) [105]. The optimal doses of 2-phenoxyethanol for the European sea bass (*Dicentrarchus labrax*) recommended to be 300 mg L^{-1}, and for gilthead sea bream (*Sparus aurata*), 450 mg L^{-1}

[25]. They found that 2-Phenoxyethanol proved to be an effective and safe anaesthetic for sea bream, *Diplodus sargus* L. and sharp snout sea bream (*Diplodus puntazzo*) [135]. It was concluded that for nutritional studies 2-Phenoxyethanol (0.2 ml L^{-1}) can be used for anesthesia for bluefin tuna *(Thunnus orientalis*) [141]. It was confirmed that rapid induction of deep anesthesia with a relatively high concentration of 2-PE (0.7 and 0.9 ml L^{-1}) for great sturgeon in hematological studies [137]. At 25°C, 2-phenoxyethanol at 0.40 g m^{-3} may be used to efficiently and safely anaesthetize vimba (***Vimba*** *Vimba, L.***)** juveniles [142].
It was found that concentrations of 0.8, 1, and 1.2 ml $L^{-1}$of 2-phenoxyethanol diluted in ethyl alcohol (1:1) in water temperatures of 22-23ºC are more suitable for short-term handling and transportation of common carp (*Cyprinus carpio*) fingerlings while 0.4 and 0.6 ml $L^{-1}$are preferable for deep sedation or the partial loss of equilibrium required for long term transportation [45]. It was found that 2-phenoxyethanol can be recommended as the most efficient anesthetics for tench (*Tinca tinca* L.) [143].

Metomiadate
Metomidate is a water-soluble powder that has been explored for use as an anaesthetic by fish biologists. It has been shown to be very effective, induction normally being achieved in 1–2 minutes with no notable hyperactivity and recovery being faster than after MS222 anaesthesia [35, 72, 81). Stress could be reduced through the use of metomidate as an anesthetic during handling as it suppress the cortisol stress response in channel catfish (*Ictalurus punctatus*) [85]. Brackish water with added metomidate used as a transport medium led to improved survival of Atlantic salmon (*Salmo salar*) smolt [38]. Metomidate may have promise in suppressing the stress response in hybrid striped bass (*Morone chrysops*×*Morone saxatilis*). It proves too useful in transporting fish since the cortisol stress response is dramatically suppressed by concentrations that allow the fish to maintain equilibrium. It proves useful in reducing cortisol and hyperglycemia often associated with handling and transportation stress in fish at low concentrations [144]. It is considered acceptable for use in culture of Senegalese sole (*Solea senegalensis Kaup 1*858) [26]. It is also found effective for use in *Oncorhynchus tschawytscha* [145, 146]. It was found that metomidate 2.0 mg L^{-1} is effective for sedation for routine weighing and measuring procedures on juveniles and 5.0 mg L^{-1} is effective for performing an ovarian biopsy or tag implantation on adults for black sea bass (*Centropristis striata*) [105].

CONCLUSION
The stress and its harmful affects due to various aquaculture activities could be mitigate by the use of anaesthetics in correct concentration. Widely used drugs for inhalation anaesthesia in fish are : MS222, clove oil, benzocaine, AQUI-S, 2-Phenoxy ethanol and metomodiate has been proven their efficacy to mitigate stress and its harmful affects due to stressors in aquaculture practices like transportation, handling, sorting by size, weighing, increased farming density, confinement level, and degradation of water quality. Though, much of the research works on stress response and efficacy of anaesthetics has been done for the marine, cool and cold water fish species. Limited researches have been done on the major cultured fresh and warm water carp fish species, especially for practical use in aquaculture.

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