Review of Literature

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a. Transportation of fishes

There is a plethora of scientific literature devoted to fish physiology and the effects of alterations in water quality, temperature, salinity, pH, ammonia, and the use of anaesthetics during transportation. Several agency and independent work groups have reviewed the efficacy of the transportation program. Transportation and handling procedures consist of several potential stressors, such as capture, on-loading, transport, unloading, temperature differences, water quality changes and stocking (Iversen et al., 1998, 2003, 2005; Finstad et al., 2003; Portz et al., 2006; Ashley, 2007). Monitoring physiological parameters during stressful operations, like transportation can provide valuable data for the establishment of adequate management practices, even for situations where there is no fish mortality (Sulikowski et al., 2005). For successful fish handling and transportation, a stronger effort towards the animal well-being is more desirable than surveying for fish mortality (Gomes et al., 2003a). The first factor of transportation is the initial health status of the fishes. Transportation of unhealthy animals may result in increased mortality during transport or after arrival at the destination.
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(Wedemeyer, 1996). Many fish are so stimulated by handling and transportation that they readily accumulate dangerous levels of lactic acid in their blood (Black, 1958). Prior to transportation, fishes may be treated prophylactically with chemotherapeutants to increase post-transport survival (Lim et al., 2003; Crosby et al., 2006b). Ideally diagnostic tests should be performed to identify and document specific pathogens before any treatment. The use of chemotherapeutants without an accurate disease diagnosis may increase production costs. In addition, inappropriate prophylactic drug treatments may harm fish. Moreover, inappropriate use of any antibiotic can increase microbial resistance (Khachatourians, 1998; Cabello, 2006).

b. Water quality

According to Portz et al. (2006), however, there are many water quality information sources for long term and intensive culture of fishes (Pickering, 1981; Adams, 2002), but sparse information related to short term holding of fish in confinement. 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 (Stefansson et al., 2007). Thermal stress occurs when the water temperature exceeds the optimal temperature range, with energy demanding stress responses, and potential decrease in individual survivorship (Elliott, 1981; Portz et al., 2006). Most fish can gradually acclimate to normal temperature changes but rapid changes in temperature, as may happen under fish loading and transportation, may result in thermal stresses or lethal conditions (Portz et al., 2006). It is well known that the excitability caused by handling, low ambient oxygen (hypoxic level and below), warm water temperature (30°C and above) increase the metabolic rates.
(rate of O₂ consumption, CO₂ output and NH₃ excretion) in aquatic animals including fishes (Peer Mohamed, 1974). Furthermore, fishes may not survive the additional oxygen demand required to sustain basal metabolism due to increased oxygen consumption from digestion and transportation stress. In addition, stress due to handling and transportation may increase oxygen consumption up to 20%. Low dissolved oxygen concentrations lead to respiratory stress, tissue hypoxia, and possible mortality (Wedemeyer, 1996).

Another consequence of metabolism is the production of carbon dioxide. As carbon dioxide levels accumulate during respiration of fishes, the pH of the shipping water declines (Wedemeyer, 1996). In addition, carbon dioxide is highly soluble and can easily diffuse across the gills, lowering the blood pH (Moran et al., 2008). The resultant blood acidosis decreases the affinity of oxygen (O₂) to bind with hemoglobin (Hb) by weakening the Hb-O₂ bond (Wedemeyer, 1996). Tissues become hypoxic when shipping water pH is lower than 6.5 and carbon dioxide levels are greater than 30–40 mg/L (Wedemeyer, 1996; Ross and Ross, 1999) which are common shipping water physico-chemical conditions. Indeed, Moran et al., (2008) reported that juvenile yellowtail kingfish *Seriola lalandi* had a 30% decrease in hemoglobin (Hb) concentration when exposed to simulated transport for 5 hours and 8 or 50 mg/L carbon dioxide. The rate of excretion of nitrogen is related to the rate of metabolism. Peer Mohamed and Devaraj (1997) reported that as with other products of metabolism, a large fish of a particular species produce less nitrogen per unit weight than do small ones. In addition, they are somewhat more resistant to the toxicity of ammonia. Gerking (1955) found that at 25°C, blue gills weighing 25 g excreted nitrogen at a rate of approximately 500 mg / kg / day, whereas the rate for fish weighing 100 g was only 120 mg / kg / day.
There are several factors that affect how fishes are packed and include time since last meal, packing materials (e.g., bags, boxes, etc.), packing density, and shipping water additives. A common practice is to withhold feed from the fishes for 1–2 days prior to transport to allow the digestive tract to be purged (Wedemeyer, 1996; Lim et al., 2003) as digestion may increase oxygen consumption by up to 50% (Wedemeyer, 1996a). This practice also aids in maintenance of shipping water quality by reducing carbon dioxide and waste production (Wedemeyer, 1996; Ross and Ross, 1999; Lim et al., 2003). Likewise the 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 (Portz et al., 2006). Therefore, optimal densities at loading and in transport tanks should always be taken care of regardless of profitability or convenience (Ellis et al., 2002; Portz et al., 2006). Optimal densities are species specific and are affected by behavioural requirements for physical space (Wedemeyer, 1996) and total length of time in transport (Lim et al., 2003). Additionally, transportation densities may be limited by potential adverse changes in water physico-chemical parameters (Lim et al., 2003). Freight is a major component of the cost of transportation of fishes (ornamental); therefore, fishes are densely packed into shipping bags with minimal water volume to reduce the overall freight weight of the shipment (Kaiser and Vine, 1998; Lim et al., 2003). Like ornamental fishes, the live fishes are frequently packed into bags with 40% water to 60% oxygen gas, but this ratio may vary depending on the species being transported (Crosby et al., 2006b).

However, with the ever-increasing variety of species being cultured for both the ornamental and food fish markets, there is no “standard” shipping
methodology that applies to all species (Emata, 2000). Nearly all aspects of fish transportation is aimed at reducing the metabolic costs of the fish while supplying the necessary elements for survival in a confined space (Durve, 1975; Weirich et al., 1992; Guo et al., 1995; Gomes et al., 2003b; Paterson et al., 2003; Colburn et al., 2008; Harmon, 2009). Fish farmers also need to be conscious of “batch variability” when it comes to transport fish, as variations in genetic makeup, feeding regime, culture conditions, or size distribution can all have marked impacts on the overall success of live fish transport. The difference between shipping success and failure typically comes down to the small variations between shipping methods and the physiological tolerance levels of the species being transported (Pennell, 1991; Weirich et al., 1992; Chow et al., 1994; Paterson et al., 2003; Pavlidis et al., 2003; Harmon, 2009).

According to Pickering (1981) these management procedures as crucial as they are, produce some level of disturbances, which can elicit a stress response leading to decreased fish performance (Maule and Shreck, 1990), alterations of the peripheral leukocyte distribution, such as heterophilia and lymphocytopenia (Ellsaesser and Clem 1986, Ainsworth et al., 1991; Gabriel et al., 2007) increased susceptibility to diseases (Pickering and Pottinger 1985; Maule et al., 1989) and in extreme, cases leads to mortality (Akinrotimi et al., 2007).

c. Stress

Transportation of fishes can be a substantial cause for stress. Stress is defined as the physiological change that occurs in response to an imposed demand on an organism that aids in the maintenance of homeostasis (Barton, 1997). Overall the stress load will affect fishes physiological
system, causing reduced growth, inhibits reproduction and suppresses its immune function. Eventually the fish will be exhausted and is likely to incur disease and die (Barton, 2002, Bonga, 1997, Barton and Iwama, 1991, Portz et al., 2006, Davis, 2010, Adams, 1990, Crosby et al., 2006). Its increased focus on stress physiology as studies show that stress has effect upon other hormones: in male songbird, the testosterone level was reduced by 37 - 52 % in response to acute stress (Deviche et al., 2010), in red-sided garter snake it was demonstrated that increased glucocorticoids inhibit melatonin synthesis (Lutterschmidt and Mason, 2010) and in rainbow trout prolactin levels were reduced up to 60% when subjected to chronic stress (Pottinger et al., 1992). The physiological stress reaction follows the same basic pattern in all vertebrates; initiated with elevated levels of catecholamine and corticosteroids. In some species concentration of corticosteroids will fluctuate with annual rhythms, as it may control other body processes (Davis and Parker, 1986). Acute stress factors such as handling and transportation cause significant increases in plasma cortisol levels, a biological indicator of stress, as demonstrated in American shad (*Alosa sapidissima*) (Shrimpton et al., 2001), coho salmon (*Oncorhynchus kisutch*) (Avella et al., 1991), rainbow trout (*Oncorhynchus mykiss*) (Woodward and Strange 1987; Pickering and Pottinger, 1989), brown trout (*Salmo trutta*) (Pickering and Pottinger, 1989), hybrid striped bass (*Morone saxatilis*, *Morone chrysops*) (Davis and Griffin, 2004), and Nile tilapia (*Oreochromis niloticus*) (Barcellos et al., 1999). The increased concentration of circulating catecholamine and cortisol will in turn cause physiological changes on blood and tissue, referred to as the secondary response. Catecholamine will cause the increased ventilation rate and blood flow for increased oxygen uptake.
and consumption, as well as initiate glycogenolysis (Portz et al., 2006). When plasma cortisol levels in fishes are elevated, blood flow and pressure are increased, oxygen demand and gill perfusion are increased, and hepatic gluconeogenesis is stimulated (Norris and Hobbs, 2006). Additionally, oxygen consumption can increase up to 20% (Wedemeyer, 1996). These physiological adaptations increase the chances of fish survival (Wedemeyer, 1996). However, studies have indicated that even small increases in plasma cortisol levels can have an immunosuppressive effect that may lead to an increased incidence of disease and mortality (Brown, 1993; Wedemeyer, 1996). Even gentle handling of fishes is a significant stress that may result in physiological changes such as an increase in plasma cortisol and blood glucose levels. Traditionally, freshwater and marine fish have been transported in both open and closed systems (Amend et al., 1982; Berka, 1986), using techniques to minimize stress and increase survival of the fish before, during, and after the transportation period (Carmichael et al., 1984; Weirich and Tomasso, 1991; Weirich et al., 1992; Gomes et al., 2003a; Harmon, 2009). In general a stressed fish will have increased metabolic rate, which gives increased load of metabolic products that in turn will give bad quality water. This is often the case in fish transported from rural communities due to the absence of equipment, holding facilities and undeveloped infrastructure in combination with poor handling techniques. To supply a resistant and healthy fish, it is necessary to establish proper handling and management that will avoid handling-related stress.

d. Anaesthetics

The use of anaesthetics has been shown to assist in the handling of fish species in aquaculture systems, reducing many of the negative impacts of
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stress (Munday and Wilson, 1997; Ross and Ross, 1999; Ortuno et al., 2002; Wagner et al., 2002; Pirhonen and Schreck, 2003). Pickering (1993) proposed sedation or mild anaesthesia as a stress-ameliorating measure during handling and transportation of fish. The anaesthetics lower the metabolic activity of fish, which facilitates the transport of more fish in a given quantity of water for a long time. In recent times anaesthetizing chemicals have been used in the transporting medium of fish seeds and adult fish. It has also been proved that anaesthetics make the otherwise time consuming work of handling, weighing, marking, tagging, fin clipping, stripping and operative procedures much easier and also lower the mortality of fish due to handling and transport (Saxena, 1986). The choice of anaesthetics is often dependent on considerations such as availability, cost-effectiveness, ease of use, nature of the study and user safety (Cho and Heath, 2000; Mylonas et al., 2005). However, the increased concern for fish health and product quality makes the use of anaesthetics inevitable to reduce the stress during handling and transportation procedures. Before recommending the use of a particular anaesthetic, a range of stress-response indices must be measured to assess its efficacy (Pramod et al., 2010). To date, much of the information on the use of anaesthetics in fish has been derived from studies on salmonids (Pickering, 1992; Iversen et al., 2003; Pirhonen and Schreck 2003; Iversen et al., 2009) and other temperate species (Mattson and Ripple, 1989). Only the liquid and solid anaesthetics, especially those, which are readily soluble in water, are useful in this field. Very little published information is available on sedation of tropical cultured fish species (Lindsay and Geddes, 1979; Basavaraja and Antony, 1997). Especially information on use of thiopentone - sodium, xylodac, lignocaine and sodium
chloride as anaesthetics/sedative agents are scanty (Saxena, 1986; Johnson and Metcalf, 1982).

Ross et al., (1993) reported that administration of anaesthetics reduced the effect of stress during handling and hauling of fish. Different handling procedures demand different anaesthetic approaches. For instance light anaesthesia (sedation), which is defined as reduced activity and reactions to external stimuli, is sufficient for procedures such as transport or weighing of fish. Full anaesthesia can be defined as loss of consciousness and reduced sensing of pain, loss of muscular tones and reflexes and is needed when surgical procedures are applied (McFarland, 1959).

Anaesthetizing the fish is often useful during handling procedures to reduce trauma and injury (Neiffer and Stamper, 2009). ‘Anaesthesia’ means loss of sensation or insensibility (Ross and Ross, 2008), and can be introduced to fish through physical or chemical techniques. Physical anaesthetics are applied through electric tension or refrigeration (Brattelid, 1999b), while chemical anaesthetics are based on immersing the fish in a water solution containing a chemical agent. These techniques will cause general anaesthesia as they affect the fish sensitivity, equilibrium and consciousness. Mostly this is introduced through ‘inhalation anaesthesia’ where the active agent mixed in the water is ventilated through the fish gills (minor through the skin). The agent will pass the blood-brain barrier and have an effect upon the fish central nervous system (CNS) (Brattelid, 1999b, Ross and Ross, 2008). The chemical agent interacts with membrane components and will cause blockage or depression of nerve impulses (Ross and Ross, 2008). This lead to loss of mobility, equilibrium and muscle reflexes (Brattelid, 1999b).
Anaesthetic treatment may reduce the fish’s perception of the stress and thus prevent the nervous input to the CNS (Woods et al., 2008, Brattelid, 1999b). This is desirable because it will block or reduce the cortisol synthesis. Cortisol elevation is known to depend upon the intensity and duration of the stressor, and may be detrimental to the fish as the cascade of physiological changes may persist for days or weeks. However, improper dosages and anaesthetic drugs may have undesirable side effects upon the fish and may self induce unnecessary stress. It is therefore necessary to find the anaesthetic and dosage that is appropriate and have desirable effects on the fish (Carter et al., 2011). An appropriate anaesthetic and the dosage will provide a smooth and rapid anaesthesia for a time period followed by recovery (Woods et al., 2008), and should not cause any undesirable side-effects. In addition, the anaesthetic agent should provide a satisfying blockage upon the hypothalamus pituitary (HPI) axis, in order to prevent cortisol elevation when anaesthesia subsides (Brattelid, 1999a).

The degree of chemical blockage upon the nervous system varies according to chemical agent, dosage and duration (Burka et al., 1997, McFarland and Klontz, 1969). McFarland (1959) was the first to classify this chemical effect into stages based on behavioural signs (Table 1). The anaesthetic effect ranged from ‘sedation’, giving a calming effect, to ‘surgical anaesthesia’, giving full immobilization. The basic procedure for introducing anaesthesia in fish is divided into three phases; introduction, maintenance and recovery (McFarland and Klontz, 1969; Ross and Ross, 2008). The depth of the introduced anaesthesia will vary according to dosage and duration. In order to not traumatize and stress the fish, the introduction phase should last for a few minutes. However, too rapid introduction is neither desirable as it
will harm and kill the fish. The most desirable anaesthesia is set to be achieved within 3 minutes (Ross and Ross, 2008; Marking and Meyer, 1985). In some procedures like transportation or surgery, it will be necessary to maintain anaesthesia. It should be kept in mind that different species will have different tolerance to dosage and duration of anaesthetic drugs. Maintenance of deep anaesthesia for a few minutes is likely to cause death from ventilation and circulatory arrest. Flaring and spasms of the opercula function as warning signals to medullary collapse (McFarland and Klontz, 1969; Ross and Ross, 2008).

### Table 1: Stages of anaesthesia; modified from (McFarland and Klontz, 1969; Burka et al., 1997)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Behaviour sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Active swimming patterns; reactive to external stimuli; normal equilibrium; normal muscle tone.</td>
</tr>
<tr>
<td>1</td>
<td>Light sedation</td>
<td>Reduced swimming activity; slight loss of reactivity to visual and tactile stimuli.</td>
</tr>
<tr>
<td>2</td>
<td>Light narcosis</td>
<td>Slightly loss of equilibrium</td>
</tr>
<tr>
<td>3a</td>
<td>Deep narcosis</td>
<td>Total loss of equilibrium; decreased muscle tone; reactivity to strong tactile stimuli; decreased respiratory rate</td>
</tr>
<tr>
<td>3b</td>
<td>Surgical anaesthesia</td>
<td>Total loss of reactivity; total loss of muscle tone; low respiratory rate</td>
</tr>
<tr>
<td>4</td>
<td>Medullary collapse</td>
<td>Respiration creases, cardiac arrest; death ensures</td>
</tr>
</tbody>
</table>

Chung (1980) classified anaesthetic effect into four different stages: first stage is where the fish is normal, reacts to external stimuli normally, swimming and opercular movements are normal. The second stage is where the fish is in a state of light anaesthesia, it becomes sluggish, has weak equilibrium, it swims partially and opercular movement is also partial while
the third stage is where the fish is in a stage of deep anaesthesia, exhibits loss of movement and very weak equilibrium with partial opercular movement. The 4th stage, which is characterized by the total loss of equilibrium, opercular movement, this in a few minutes leads to heart failure. The second and third stages are of great relevance as the fish is then insensitive to pain. The choice of anaesthetics for fish must be based on the species, the size of fish and the duration of operation, water temperature and chemistry, exposure time, good safety margin (Lemm, 1993). The time to introduce anaesthesia depends on both biotic and abiotic factors. Age, lipid content, size and metabolism are biological factors that will affect the anaesthetic effect. The anaesthetic can also have different effects within the same species due to biological differences like sex, life-stage and season (Brattelid, 1999b).

Recovery from anaesthesia will occur when the fish is immersed in freshwater. The anaesthetic agent is then excreted through the gills. As with the introduction of anaesthesia, recovery is also divided into different stages based on behavioural sign (Table 2). The recovery should be attained within few minutes to prevent stress and harmful effects on the fish (Woods et al., 2008). The most desirable recovery is set to be retained within 5 minutes (Marking and Meyer, 1985; Ross and Ross, 2008). Higher concentrations and longer exposure time of the anaesthetic correspond with longer recovery time (McFarland and Klontz, 1969). After anaesthetic procedure the fish is recommended to be under closer observation for 24-72 hours, as death can occur (Ross and Ross, 2008).
Table 2 Stages of recovery; modified from (Hikasa et al., 1986)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Behaviour sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reappearance of opercula movement; weak muscle tone visible</td>
</tr>
<tr>
<td>2</td>
<td>Reappearance of swimming activity, but still loss of equilibrium</td>
</tr>
<tr>
<td>3</td>
<td>Partial recovery of equilibrium</td>
</tr>
<tr>
<td>4</td>
<td>Full recovery of equilibrium; reaction in response to visual and tactile stimuli; still stolid behavioural response</td>
</tr>
<tr>
<td>5</td>
<td>Total behaviour recovery; normal swimming activity</td>
</tr>
</tbody>
</table>

Use of anaesthetic is well established within the aquaculture sector for food fish during handling, transport, confinement, vaccination, grading, etc. There are several different chemical drugs that can immobilize fish, but not all are described as safe and effective for use on fish. However, the wide variety in anatomy, physiology and behaviour in the fishes, make the anaesthetic treatment potential harmful (Neiffer and Stamper, 2009), however, there are some publications which emphasize on the anaesthetic efficacy on some species (Bircan- Yildirim et al., 2010; Young, 2009; Grush et al., 2004; Kaiser and Vine, 1998). Marking and Meyer (1985) listed up six criteria for an ideal anaesthetic; permit the reasonable duration of exposure, produce anaesthesia within 3 minutes or less, allow recovery within 5 minutes or less, cause no toxicity to fish at treatment levels, present no mammalian safety problems and leave no tissue residues after a withdrawal time of 1 hour or less.

The chemical properties of anaesthetics may depend upon environmental factors like temperature, pH, salinity, chemical additives and oxygen content (Burka et al., 1997). Lipid soluble anaesthetics may depend upon temperature or solvent for resolution, and some anaesthetic will in turn have effect upon water parameters. Fish is a poikilotherm animal and temperature will affect
its biological functions. Both temperature and pH will affect gill perfusion, which in turn affects uptake and clearance rate of the anaesthetic agent (Ross and Ross, 2008; Burka et al., 1997). To avoid undesirable effects on the fish, the anaesthetic treatment is recommended to be carried out in water close to the fish biological optima (Brattelid, 1999b).

There are a variety of anaesthetic agents such as tricaine methane sulfonate (i.e., MS-222), quinaldine, metomidate hydrochloride, and clove oil that has been used in shipping water to alleviate transportation related stress; however, it is important to note that there are no drugs currently approved by the U. S. Food and Drug Administration (FDA) for transporting fishes. The shipping water may be treated such that the fishes are shipped under sedation, a stage of anaesthesia. During transport, anaesthetics should only lightly sedate fish, not anaesthetize them, to avoid interfering with osmoregulation or gas exchange (Forteath 1993). The stress response may be minimized if the anaesthetic takes effect quickly (Robertson et al., 1988; Ross and Ross 1999). Tricaine Methanesulfonate (TMS) is absorbed by the fish and its effects are cumulative over time (Crosby et al., 2006c). Additional TMS may need to be added to sedate all fish (Brown, 1993), but too much TMS may over anaesthetize fish, leading to ventilatory arrest (Ross and Ross, 1999). Metomidate has been shown to suppress parts of the biochemical pathway blocking cortisol synthesis (Ross and Ross, 1999). Quinaldine is inexpensive, effective, and undetectable 24 h after exposure; it is more potent at for sedation higher temperatures and in hard water (Ross and Ross, 1999). Hypno is a proprietary registered product for sedation containing quinaldine (Crosby et al., 2006c). However, there may be problems associated with the use of these anaesthetic agents. For example, sedation with MS-222 or
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Quinaldine may cause an initial excitatory response that results in increased plasma cortisol levels; and clove oil have a slow induction time (Barton and Peter, 1982; Robertson et al., 1987; Ross and Ross, 1999). Hypothermia (cold anaesthesia) known to reduce the stress in fish handling, either by itself or in combination with chemical anaesthetics (Rodman, 1963; Hovda and Linley, 2000; Ross and Ross 1999). Yoshikawa et al. (1989) showed that carp, previously acclimated to 23°C, would be safely held at 5 °C for 5 h, and achieved sedation at 8-14° C for 24 h.

Clove oil is the best-known herbal product used as a local analgesic and it has long been employed to obtain transient relief from toothache (Ghelardini et al., 2001). In Indonesia, it has been used as a topical anaesthetic for tooth aches, headache and joint pain (Soto and Burhanuddin, 1995). Clove bud oil, which obtained by distillation method is a clear, colorless to yellow mobile liquid, becoming browner with age or contamination with iron or copper, with a strong characteristic sweet and spicy clove odor, and a warm, almost burning and spicy flavor (Weiss, 1997). Moreover, this oil consists of three components: eugenol (70-90%), eugenyl acetate (17%) and caryophyllene sesquiterpenes (mainly β-caryophyllene) 5 – 12% (Vernin et al., 1994). The other constituent of clove bud oil is β-caryophyllene (Walter, 1972). This component has a local anaesthetic activity similar to eugenol as reported by Ghelardini et al. (2001). They compared β-caryophyllene with caryophyllene and found that the former has a strong local anaesthetic action when administered in rabbits. Clove oil has been used as a mild anaesthetic since antiquity and its effectiveness as an anaesthetic in dentistry is well known (Ross and Ross, 1999). Clove oil is readily available and is inexpensive compared to MS-222 (Keene et al., 1998). The primary constituent
of clove oil, eugenol, is similar in structure to Tricaine metanesulfonate and 2-phenoxyethanol (Varner, 2000). The anaesthetic effects of eugenol have been studied to varying degrees on *Medaka oryzias latipes* (Temminck and Schlegal, 1846), gold fish *Carassius auratus* (L.) and crucian carp *C. carassius* (L.) (Endo et al., 1972 as cited by Keene et al., 1998). Hikasa et al., (1986) showed that it gave effective anaesthesia in adult common carp (*Cyprinus carpio*) at 25 to 100 ppm. Soto and Burhanuddin (1995) studied the use of clove oil as a tool of sedation for measuring length and weight of rabbit fish (*Siganus lineatus*).

Cinnamon (*Cinnamomum zeylanicum*) which is native to India and Sri Lanka (Ceylon) Vaibhavi and Jakhetia et al., (2010) and now it is cultivated in many tropical countries, including Mexico as one of the most important medicinal plants. Cinnamon contains 0.5 to 1.0% volatile oil composed mainly of cinnamyldehyde (50.5%), eugenol (4.7%), cinnamic acid, methoxycinnamaldehyde (MOCA) and cinnamyl acetate (8.7%) (Charu Gupta et al., 2008). Research interest has focused on the cinnamon that possesses antispasmodic, anti-ulcer, sedative, hypothermic, antifungal, antibacterial, antiviral, antipyretic, lipolytic, anaesthetic, cytotoxic, hypolipidemic, antiplatelet properties and also stimulates the immune system that may be useful adjuncts in helping to reduce the risk of cardiovascular disease and cancer (Cralg, 1999). Cinnamon (*Cinnamomum zeylanicum*) bark also contains eugenol, but its use as an anaesthetic has not been explored (Power et al., 2010). Eugenol content of the leaf oil is antiseptic and anaesthetic (Khare, 2007).

Tobacco is the common name for the plant *Nicotiana tobacum*. It is a native of tropical and subtropical America, but it is now commercially
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cultivated worldwide (Knapp, 2004). Tobacco contains the following phytochemicals: Nicotine, Anabasine (an alkaloid similar to the nicotine but less active), Glucosides (tabacinine, tabacine), 2,3,6-Trimethyl-1,4-naphthoquinone, 2-Methylquinone, 2-Naphthylamine, Propionic acid, Anatalline, Anthalin, Anethole, Acrolein, Anatabine, Cembrene, Choline, Nicotelline, Nicotianine and Pyrene and they are generally recognized as being narcotic (Agokei and Adebisi, 2010). This property makes it useful as narcotics, mulluscicides, piscicides, an anaesthetic and pesticide (Aleem, 1983; Agbon et al., 2002). Agokei and Adebisi (2010) reported that the tobacco extracts acted as an anaesthetic in Nile tilapia, *Oreochromis niloticus*. Detailed studies on the use of tobacco as an anaesthetic for juveniles of *Etroplus suratensis* is not available and it would appear that experimental studies on this subject are rare.

The most common synthetic anaesthetic agent used on fish is Tricaine Methanesulfonate (MS-222) (Marking and Meyer, 1985) and is the only anaesthetic verified by the U.S. Food and Drug Administration (FDA). It occurs as a white crystalline powder directly applied to the water. However, this anaesthetic agent is regarded as a carcinogen and also a 21-day withdrawal period is required if the fish is intended for human consumption (Kolanczyk et al., 2003; Rombough, 2007). Additionally, MS-222 is relatively expensive and frequently unavailable in many countries due to several international restrictive rules regarding import of this chemical. Although a number of studies have described the physiological responses of fish to sedate and immobilizing doses of MS-222, only a few studies have reported on responses to higher, lethal concentrations of MS-222 or other anaesthetics. A few studies using higher concentrations of MS-222 were 125 mg L⁻¹ (Laidley and Leatherland, 1988); 150mg L⁻¹ (Holloway et al.,
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2004) have evaluated changes in blood chemistry at the time of induction of deep anaesthesia, 2-3 min after the initiation of exposure.

In recent years, a shift in research has occurred where response to cold shock is measured in terms of sub lethal effects to fishes rather than just mortality. The cold-shock response may be a beneficial tool for fisheries science (e.g. for induction of polyploidy) and future cold-shock research may reveal other novel opportunities. Hypothermia is also known to reduce the stress in fish handling, either by itself or in combination with chemical anaesthetics (Rodman, 1963; Hovda and Linley, 2000; Ross and Ross, 1999). Application of hypothermia or cold shock has been reported as a short-term anaesthetic (Hovda and Linley, 2000), as a means to alter embryonic sex ratios (Craig et al., 1996) and, most commonly, as an agent in the induction of polyploidy (Peruzzi et al., 2007). The basic principle behind the hypothermia for live transportation of fishes is cold temperature induced anaesthetization. The water temperature is brought down to a limit at which the metabolic rate of the animal is reduced to a minimum, so that its storage and transport in this condition does not affect any apparent increase in metabolic rate. Yoshikawa et al., (1989) showed that carp, previously acclimated to 23°C, would be safely held at 5°C for 5 h, and achieved sedation at 8-14°C for 24 h. The movements of the cold-anaesthetized shrimp are minimum, there is no stress caused by vibrations, noise and light; weight loss is usually negligible, and the animals produce no excreta because there is no feed intake and metabolism (Schoemaker, 1991).

The present work attempts to assess the stress reducing capacity of certain anaesthetics such as clove oil (*Syzygium aromaticum*), cinnamon oil
(Cinnamomum cassia), Cassumunar Ginger (Zingiber cassumunar) extract, tobacco leaf (Nicotiana tobaccum) extract, MS-222 (Tricaine methanesulphonate) and cold (hypothermia) anaesthesia on the experimental organism selected for this study, namely Green chromide (Etroplus suratensis, Bloch, 1790), and to probe into the behavioural, toxicological, haematological and biochemical responses of the organism.
1.1 Introduction

The toxicity of certain plant extracts on fish has been reported (Ufodike and Omorogie, 1994; Onusiruika and Ufodike, 1994; Aguigwo, 1998). The toxicity and effect of anaesthetics are of special interest since they are frequently used in research and routine aquaculture procedures to immobilize fish and minimize their stress responses (King et al., 2005). Toxicity refers to the degree at which a substance is being harmful, destructive or poisonous to life (Boyd and Lichtkoppler, 1979). There are numbers of terms that are...