Aquaculture is the fastest growing food-producing sector worldwide and has recently been awarded increasing interest and priority in India. However, an increased awareness has grown among the public, as well as government, of the need to secure the welfare and health of farmed fish. For example, a major current challenge for the industry is to find ethically acceptable methods to handle, immobilize during transportation from rearing site to farm. A bottleneck in the continued development of live fish transportation techniques and refinement of existing techniques has been to find reliable physiological markers of stress in fish in order to identify and quantify steps in these processes that may cause stress and suffering and thereby lead to impaired welfare.

The present study is specifically focused on one of the most important aquaculture species; Green chromide (*Etroplus suratensis*) the state fish of Kerala. In order to assess the welfare of juveniles of Green chromide (*Etroplus suratensis*) during commercial transport from hatchery to farm site, it is necessary to reduce stress responses in fish. For this purpose, we have primarily used selected anaesthetics (clove oil, cinnamon oil, cassumunar ginger extract, tobacco leaf extract, MS-222 and cold) during 24-48 hours...
commercial transportation enabling sufficient survivability. The objectives in this chapter proved the importance of plant anaesthetics by scrutinize its toxicity which is capable to control the erratic behavioural, haematological and biochemical stress indices and thereby increase the water quality of the packing system. Here also specifies the importance of physical anaesthetic (hypothermia) which also controls the stress indices for the maximum packing density.

The present study on toxicological effects of clove oil, cinnamon oil, cassumunar ginger extract, tobacco leaf extract, MS-222 and cold on juveniles of *Etroplus suratensis* were conducted under laboratory conditions using the static bioassays and continuous aeration. The aim was to develop an effective anaesthetic from an indigenous plant material that will be available at low cost to aqua culturists and which would be non-toxic to the fish and consumers. The active ingredients and their compositions in the plant anaesthetics (clove oil, cinnamon oil, cassumunar ginger extract, tobacco leaf extract) as well as chemical (MS-222) and physical anaesthetics (hypothermia) during 96 hours acute toxicity (LC$_{50}$) on juveniles of *Etroplus suratensis* were investigated. Data obtained from this investigation were subjected to 95% confidence intervals and analysis of variance (ANOVA) at 0.05 level of significance. No mortality was observed in the present study group exposed to lower concentrations of selected anaesthetics within the first 24 hours of exposure. For the selected anaesthetic concentrations tested in this experiment, the mortality rate was always higher at higher concentrations during 96 h duration. Depending on the duration of hours, the mortality rate at each concentration differed. The cumulative mortality rate indicated that mortality rate of the test fish and concentrations of selected anaesthetics are
positively correlated. During each exposure period (24, 48, 72 and 96 hours) of the acute toxicity test for selected anaesthetics, it was observed that the mean values of all water quality parameters were significantly different ($P < 0.05$). This study has shown that clove oil is less toxic, highly effective, cost efficient and safe anaesthetic for juveniles of *Etroplus suratensis* use in aquaculture and laboratory research settings at concentrations not more than 0.10 mg L$^{-1}$.

The present study reveals that the use of low concentrations of certain plant extracts and oils (clove oil, cinnamon oil, cassumunar ginger extract, tobacco leaf extract) that shows anaesthetic property to achieve sedation through behavioural assessments for fish handling and transportation, compared to the synthetic anaesthetic (MS-222) and physical (hypothermia) anaesthetic was also evaluated along with plant anaesthetics. Clove oil gave the best induction and recovery times. Cinnamon oil compared with clove oil had a significantly longer time to sedation. Cassumunar Ginger (*Zingiber cassumunar* Roxb) showed some anaesthetic properties but was less effective due to longer induction and recovery times. Tobacco leaves (*Nicotiana tobaccum*) showed some anaesthetic properties but were toxic. It is concluded that anaesthetic properties of clove are comparable with the recommended criteria for being an effective anaesthetic. Although MS-222 (tricaine methanesulphonate) is an effective fish anaesthetic with the desirable characteristics of rapid induction and recovery times, but it has less margin of safety and also has 21 days of withdrawal period. The study also proved that application of hypothermia in *Etroplus suratensis* would result anaesthetization within a shorter time in minimal signs of distress. During transportation the induction and recovery rate and the fresh condition of the
live fish indicate the good physiological parameters, demonstrating the benefits of live transportation of fishes.

Behavioural assessments through video monitoring systems are becoming regular in live fish transport. Video monitoring systems is a convenient and non-invasive tool for assessing the behaviour of transported fish; therefore in the present work attempted to come up with easily measured criteria that were comparable to physiological measurements. It is important to determine, however, what the behaviour is for a given group of juveniles as there can be differences in behaviour depending upon strain and rearing conditions.

Results of the present investigation revealed certain haematological parameters (Hct, Hb, RBC, MCV, MCH, PCV and MCHC) in the blood of *Etroplus suratensis*, during prolonged exposure (24 and 48 h) to the optimum concentrations of clove oil, cinnamon oil, cassumunar ginger extract, tobacco leaf extract, MS-222 and cold (hypothermia) showed significant difference with the control group.

As predicted, primary stress (as assessed by plasma cortisol values) was greatest immediately following truck transport. However, primary and secondary stresses were only moderate when compared with other work cited on fish and stress physiology. Because stress responses immediately before and during harvest have been shown to affect product quality (Robb 2001), one can assume anything that increases the stress response before harvest will likely have a similar increased negative effect on the end product quality and therefore should be avoided if possible. Among the anaesthetic treatments investigated in the present study (clove oil, cinnamon oil, cassumunar ginger...
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extract, tobacco leaf extract, MS-222 and cold) the cassumunar ginger extract and tobacco leaf extract were not effective in consistently reducing the stress responses of juveniles of *Etroplus suratensis* during the 24-48 h simulated transport. All treatment groups showed stress responses for each of the parameters measured. Use of clove oil during 24-48 h transport indicated no clear advantage over water quality. Treatment with cinnamon oil does not give good margin of safety, and thus its use cannot be justified. Hypothermia treatment actually indicated less stress responses compared to other treatment, as indicated by significantly lower cortisol, glucose, and lactate responses. Use of MS-222 resulted in more severe stress responses (haematocrit and cortisol) compared to other treatments and control. But during the combinations of clove oil and hypothermia, it likely indicates a deleterious rather then beneficial effect on end product quality after 24-48 h transportation. Overall, use of anaesthetics during transport of juveniles of *Etroplus suratensis* provided clear advantage, and in the case of combination of anaesthetics viz., clove oil with hypothermia (0.10 mg l\(^{-1}\) at 18°C) may have resulted in additional gain of product quality because of a less stress response.

Although all six anaesthetic agents evaluated were effective and presented a good margin of safety, clove oil with hypothermia proved to be most effective in the juveniles of *Etroplus suratensis*. Due to the investigation of different anaesthetic induction and recovery stages, as well as the identification of the lowest effective concentrations of each anaesthetic agent, the findings of the present study has potential significance with regards to pearl spot (*Etroplus suratensis*) husbandry, stress, survival, transport and revenue. In particular the chemical anaesthetic are not reachable for invasive
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fisheries research procedures and aquaculture procedures due to the high cost, in availability, hardy to use, lethal to fish and less margin of safety.

Based upon the positive results of our study using clove oil with hypothermia to transport *Etroplus suratensis* coupled with the growing body of literature cited we suggest that clove oil with hypothermia should be an effective alternative transport anaesthetic. Our study focused on the use of clove oil with hypothermia for fish transportation. The concentrations required to induce anaesthesia identified as optimal for fish transport should also be effective for the general handling of cultured fish for grading, marking, enumerating, inspection, and gamete stripping. This study is the first to identify euthanasia methods for *Etroplus suratensis*, (as described by American Veterinary Medical Association [Internet] (2007) AVMA guidelines on euthanasia, 2007) and the outcome will be important in assisting institutional animal care and use committees and researchers in determining of the most appropriate method of euthanasia for *Etroplus suratensis*. Further work will also be needed to determine its utility for large-scale operation. The results of this study comprise a refinement to *Etroplus suratensis* euthanasia techniques and provide more information on techniques necessary for *Etroplus suratensis* studies for the laboratory animal and biomedical research community. The work described here has, for the first time, provided a systematically derived system of safe, long distance, live transportation of this species and others of the genus *Etroplus*. These species are delicate and easily stressed and this system opens further the opportunities for their culture during in large scale operation.