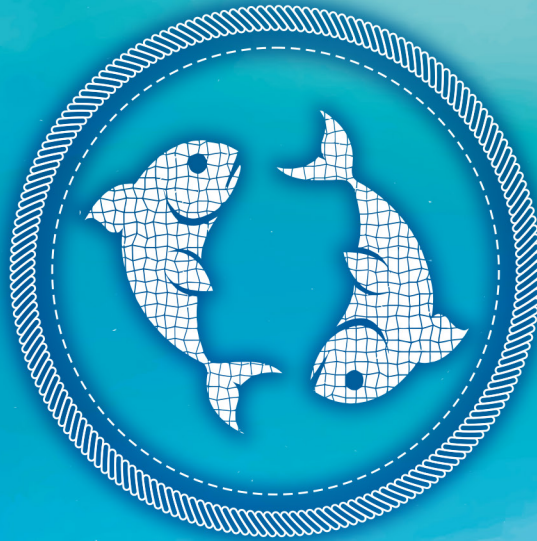


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## STRESS INDUCED BY DIFFERENT SLAUGHTERING METHODS IN GILTHEAD SEABREAM

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### Abstract

The purpose of this research was to investigate and compare stress response induced by four slaughtering methods (asphyxia, bleeding, immersion in ice-water and over-dose of anesthetics (phenoxyethanol, *Eugenia aromatica* and *Cinnamomum zeylanicum*) in gilthead sea bream. Stress responses were determined by detecting fragmented DNA in hepatocytes using the molecular technique, single-cell gel electrophoresis (Comet Assay), which can detect primary DNA damage in individual cells. Presently, slaughtering methods without the presence of anaesthetics increased the level of stress in fish. Overdose of anesthetics (phenoxyethanol and essential oils) recorded significantly lower TM values compared to asphyxia, bleeding and immersion in ice-water. No significant differences were recorded between the three applied anaesthetic agents.

**Keywords:** *welfare, slaughtering methods, sea bream, stress, comet assay.*

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### 1. Introduction

Ethical and welfare issues concern the aquaculture industry since animal welfare has become an important factor for the consumer attitudes towards aquaculture products. According to the Scientific Panel on Animal Health and Welfare of the European Food Safety Authority, stunning/slaughtering procedure is one of the critical steps that affect both quality and welfare. In order to minimize the suffering during slaughter, the use of methods that make fish less sensitive to stressful procedures prior to the actual death should be applied. Various anaesthetizing methods meet this demand and do not negatively affect the final quality of fish (Ribas et al., 2007). However, as anaesthetic agents are based on chemicals, they should be rejected considering the potential risk for human consumption, while it is important to continue the research on stunning methods alternative to chemical anesthetics. Besides the ethical considerations and therefore to avoid fish suffering, there are also economical and commercial reasons, since quality in badly treated fish is reduced. It is well known that inadequate handling before the slaughtering may lead to faster flesh deterioration (Lowe et al., 1993, Sigholt et al., 1997).

Reliable assessment of animal welfare and quality requires the study of stress indicators. The purpose of this research was to investigate and compare stress response induced by four slaughtering methods (asphyxia, bleeding, immersion in ice-water and over-dose of anesthetics (phenoxyethanol, *Eugenia aromatica* and *Cinnamomum zeylanicum*) in gilthead sea bream *Sparus aurata* L. Stress responses were determined by detecting fragmented DNA in hepatocytes using the molecular technique, single-cell gel electrophoresis (Comet Assay), which can detect primary DNA damage in individual cells. The study will present information on how the usual husbandry methods could affect welfare and quality in cultured fish.

### 2. Material and Methods

Stress response induced by different slaughtering methods was assessed in gilthead seabream. Fish was acclimated for a month in 150L tanks. Throughout the experiment, the water temperature was stable at 21±1°C. Fish were fed daily with commercial pellets, while no mortalities were observed. Four slaughtering methods were applied, asphyxia, bleeding, immersion in ice-water and over-dose of three anesthetics (phenoxyethanol and two essential oils *Eugenia aromatica* and *Cinnamomum zeylanicum*). High dose (0.25%) was presently applied in case of the anaesthetic agent phenoxyethanol. The essential oils *Eugenia aromatica* and *Cinnamomum zeylanicum* known for their anaesthetic properties were also applied at high doses (500ppm) dissolved in 95% ethanol at ratio 1:10 (essential oil:ethanol). For each treatment, three replicates were applied.

Stress levels were determined by detecting fragmented DNA in hepatocytes using comet assay (parameter: TM-Tail Moment). The hepatocytes were isolated according to Baksi & Fazier 1990, Devaux *et al.* 1997, Mitchelmore & Chipman 1998 and lysis and cell electrophoresis were then performed. For slide staining Ethidium Bromide (EtBr) was used. On each slide, 50 cells were randomly picked. Images were captured by a high-resolution video camera and projected on a PC screen. The data was analyzed by the Comet Assay Software Project (CASP) and genotoxicity TM data, were expressed as the mean±standard error and analyzed using one-way ANOVA followed by Tukey post hoc test.



### 3. Results

As shown in Figure 1, significantly higher TM values were recorded when asphyxia, bleeding and immersion in ice-water were applied as slaughtering methods, compared to overdose of anaesthetics ( $P < 0.05$ ). Asphyxia, bleeding and immersion in ice-water do not present statistically differences ( $P > 0.05$ ). Furthermore, no significant differences were recorded between the three applied anaesthetic agents.

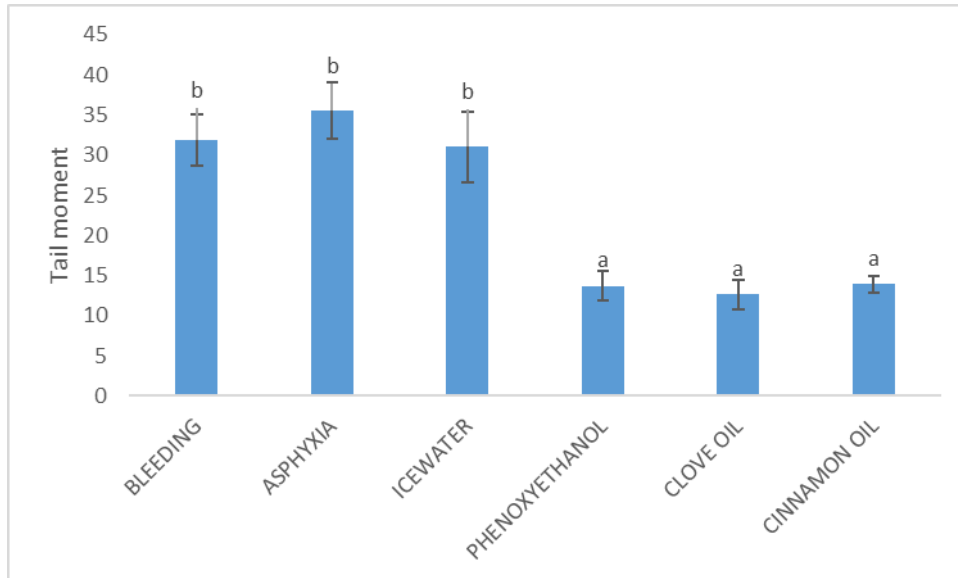


Figure 1. Tail Moment values in hepatocytes (Vertical bars represent standard errors)

### 4. Discussion

The animal welfare during production has always been an important issue. Recently, there has also been an increased awareness on suffering during the final slaughtering phase. Reviews on slaughtering methods for fish (Robb *et al.*, 2000) presently address questions about the possibility of ethical considerations in industry procedures for slaughtering (Lambooij *et al.*, 2002). Slaughtering methods should lead to a rapid and irreversible loss of consciousness, product quality and human safety. Humane slaughter procedures, can also improve *post mortem* quality of fish, as reported for warm-blooded animals (Brown *et al.*, 1998, Geesink *et al.*, 2001). Selection of appropriate slaughtering method depends on the fish species and its physiological functions (Morzel *et al.*, 2003).

Presently, stress response induced by different slaughtering methods was assessed in gilthead seabreams (*Sparus aurata* L.). Four slaughtering methods were applied, asphyxia, bleeding, immersion in ice-water and over-dose of three anesthetics (phenoxyethanol and two essential oils *Eugenia aromatica* and *Cinnamomum zeylanicum*). In all cases slaughtering methods without the presence of anaesthetics increased the level of stress in fish. Overdose of anesthetics (phenoxyethanol and essential oils) recorded significantly lower TM values compared to asphyxia, bleeding and immersion in ice-water. Asphyxia and immersion in ice-water are proven to be stressful killing methods (Ribas *et al.*, 2007, Acerete *et al.*, 2009). However, immersion in ice-water, is the most widely used killing method in fish farming combining easiness, bulkiness and the low cost. Use of methods that make fish less sensitive to stressful procedures prior to the actual death should be applied, in order to minimize the suffering during slaughter. According to Weber *et al.* (2009) phenoxyethanol causes the fastest anesthesia which means the faster loss of consciousness of fish. Furthermore, essential oils are an alternative option for fish anesthesia, which is necessary for well-being in intensively farmed conditions (Golomazou *et al.*, 2016). They are used as anesthetics and combine low cost, easy access, efficacy and safety for the environment and the user (Akbulut *et al.*, 2010). However as effectiveness and genotoxicity of essential oils are dose depended (Slamenova and Horvathova, 2013), more research has to be done in order to be assessed their safety for the cosumers.

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