

## Effectiveness of eugenol sedation to reduce the metabolic rates of cool and warm water fish at high loading densities

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### Abstract

Effects of eugenol (AQUI-S<sup>®</sup> 20E, 10% active eugenol) sedation on cool water, yellow perch *Perca flavescens* (Mitchill), and warm water, Nile tilapia *Oreochromis niloticus* L. fish metabolic rates were assessed. Both species were exposed to 0, 10, 20 and 30 mg L<sup>-1</sup> eugenol using static respirometry. In 17°C water and loading densities of 60, 120 and 240 g L<sup>-1</sup>, yellow perch controls (0 mg L<sup>-1</sup> eugenol) had metabolic rates of 329.6–400.0 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>, while yellow perch exposed to 20 and 30 mg L<sup>-1</sup> eugenol had significantly reduced metabolic rates of 258.4–325.6 and 189.1–271.0 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> respectively. Nile tilapia exposed to 30 mg L<sup>-1</sup> eugenol had a significantly reduced metabolic rate (424.5 ± 42.3 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) relative to the 0 mg L<sup>-1</sup> eugenol control (546.6 ± 53.5 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) at a loading density of 120 g L<sup>-1</sup> in 22°C water. No significant differences in metabolic rates for Nile tilapia were found at 240 or 360 g L<sup>-1</sup> loading densities when exposed to eugenol. Results suggest that eugenol sedation may benefit yellow perch welfare at high densities (e.g. live transport) due to a reduction in metabolic rates, while further research is needed to assess the benefits of eugenol sedation on Nile tilapia at high loading densities.

**Keywords:** sedatives, eugenol, respirometry, metabolic rate, fish transport, loading density

### Introduction

Minimizing metabolic disturbance when fish are held at high densities (e.g. live transport) can be challenging for fish culturists and is important for the welfare and survival of fish (Harmon 2009). Measuring metabolic rates of fish can be used as an indicator for the quality of the culture or transport environment (Cech 1990). Specifically, changes in metabolic rate can indicate degrading water quality, stress and physical injury. Metabolic rate (e.g. oxygen consumption) is typically measured in respirometry systems during periods of rest, routine swimming or elevated swimming (Beamish 1963).

During intensive culture or fish transport, oxygen consumption can increase due to stress associated with tank loading (Wedemeyer 1972), high loading densities (De Abreu, Sanabria-Ochoa, Goncalves & Urbinati 2008) and decreased water quality (Hill & Forster 2004; Carneiro, Kaiseler, Swarofsky & Baldisserotto 2009). Increased oxygen consumption is a secondary response to a physical or chemical stressor (Barton & Iwama 1991; Cho & Heath 2000; Portz, Woodley & Cech 2006) and chronic or multiple acute exposures to a stressor can result in a compromised immune system and ultimately mortality (Wendelaar 1997). Mortality resulting from the stress of high loading densities (i.e. transport) has been documented up to 14 days post transport (Ross & Ross 2008).

To mitigate stressors, sedatives have been studied as an additive during transport of live fish (Cooke, Suski, Ostrand, Tufts & Wahl 2004). Tricaine-methanesulfonate [3-aminobenzoic acid ethyl ester methanesulfonate (MS-222)] is the only sedative approved for finfish use in the United States. MS-222, an efficacious immobilizing agent (Trushenski, Bowker, Gause & Mulligan 2012), is restricted in the United States by the 21-day withdrawal period assigned by the US Food and Drug Administration (FDA). For fish transport, there is a strong need for a short (<1 day) withdrawal sedative that can be used in aquaculture and by fisheries managers (Bowker & Trushenski 2012). Currently, AQUIS<sup>®</sup>20E (10% active eugenol; AQUIS New Zealand Ltd., Lower Hutt, New Zealand) is being evaluated by the FDA to sedate fish to handleable followed by immediate release to freshwater.

Eugenol (2-methoxy-4-prop-2-enyl-phenol) is the primary active ingredient in clove oil (70–90% by weight) and has been heavily researched as a general sedative of fish (Hoskonen & Pirhonen 2004; Roubach, Gomes, Fonseca & Val 2005; Palic, Herolt, Andreasen, Menzel & Roth 2006; Park, Im, Seol & Park 2009). Results of various studies using clove oil have shown short induction and recovery times (Anderson, McKinley & Colavecchia 1997; Gomes, Chaves, Becker & Baldisserotto 2011), low physiological disturbances (Cho & Heath 2000), rapid absorption and elimination from tissues (Kildea, Allan & Kearney 2004; Guenette, Uhland, Helie, Beaudry & Vachon 2007) and the ability to minimize primary and secondary stress responses (Iverson, Finstad, McKinley & Eliassen 2003; Deriggi, Inoue & Moraes 2006; Palic *et al.* 2006). Although eugenol in the raw form of clove oil has been evaluated for multiple properties of sedation on individual fish, there is a gap of information concerning how high densities of fish respond to sedation. Furthermore, few studies have assessed a product where eugenol is the only active ingredient, which is important due to the potential carcinogenic properties of the additional active ingredients of clove oil (USDHHS 2007).

Yellow perch *Perca flavescens* (Mitchill) and Nile tilapia *Oreochromis niloticus* L. are two economically important fish and were used as model cool and warm water species to monitor metabolic rates. Worldwide, production of fish categorized under the common name tilapia generated over \$3.7 billion USD in 2009 (FAO 2009). In the United States, Nile tilapia are predominantly cultured

as a food fish, while yellow perch are raised for both stocking (i.e. recreational harvest) and as a food fish (Malison 2000; El-Sayed 2006). Nile tilapia is robust to hypoxic environments, which has promoted their success in culture worldwide (Abdel-Magid & Babiker 1975). However, low dissolved oxygen (i.e. <3.5 mg L<sup>-1</sup>) has been shown to negatively impact the survival of yellow perch during intensive and extensive culture (Malison 2000).

The objective of this study was to examine the effectiveness of eugenol to reduce the metabolic rates of yellow perch and Nile tilapia held at high loading densities. Specifically, identifying eugenol concentrations that reduced metabolic rates would provide fish culturists with a tool to promote the welfare of fish held at high densities.

## Materials and methods

### Study animals

Yellow perch [9.7 ± 4.5 g wet weight, 10.1 ± 1.3 cm total length (TL)] were hatched in outdoor ponds and reared in 17°C flow-through tanks at the Upper Midwest Environmental Sciences Center [(UMESC), La Crosse, WI, USA]. Nile tilapia (25.9 ± 7.6 g wet weight, 11.4 ± 1.3 cm TL) were obtained from a commercial farm in Florida, which administered feed containing 17 $\alpha$ -methyltestosterone to the fry to produce a population of >80% phenotypic males before shipment to UMESC. Nile tilapia were then reared at UMESC in 24°C flow-through tanks. After the grow-out phase at UMESC, yellow perch and Nile tilapia were then transported in truck tanks to the aquaculture facility at the University of Wisconsin – Stevens Point (Stevens Point, WI, USA). Yellow perch were held in 2800 L and Nile tilapia in 1890 L recirculating aquaculture systems (RAS) and fed daily maintenance rations of ~1% body weight (BW) per day. Yellow perch were fed an extruded salmon diet [slow-sinking crumbles (1.6 mm); Skretting USA, Tooele, UT, USA] and Nile tilapia were fed a tilapia diet [extruded pellets (6.4 mm); Purina Mills, St. Louis, MO, USA]. Fish were not fed 12–15 h prior to eugenol exposure.

### Loading density calculations

Multiple loading densities of yellow perch and Nile tilapia were exposed to multiple eugenol

concentrations to determine effects on metabolic rates. The total flask volume of 1.1 L and water displacement of 1.0 mL g<sup>-1</sup> BW were accounted for in loading density calculations. Initially, yellow perch were to be tested at higher loading densities of 120, 240 and 360 g L<sup>-1</sup> and Nile tilapia at 240, 360 and 480 g L<sup>-1</sup>. Due to the conical shape of the flask, it was determined during pilot trials that yellow perch at 360 g L<sup>-1</sup> and Nile tilapia at 480 g L<sup>-1</sup> would be an unreasonable density to fit inside the chamber. Therefore, yellow perch were tested at target loading densities of 60, 120 and 240 g L<sup>-1</sup> and Nile tilapia at 120, 240 and 360 g L<sup>-1</sup>. Fish weights were later used to calculate the exact loading densities tested. In addition, to further explore the effects of eugenol below the three previous loading densities, a low loading density of ~20 g L<sup>-1</sup> (yellow perch) and 40 g L<sup>-1</sup> (Nile tilapia) was tested. This loading density was substantially lower than the three previous densities and the results were important for assessing the effects of eugenol on metabolic rates when sedating low densities of fish (e.g. general sedation).

#### AQUI-S<sup>®</sup>20E calculation

The mass of AQUI-S<sup>®</sup>20E required to make the stock solutions (e.g. 10 mg L<sup>-1</sup> eugenol = 100 mg L<sup>-1</sup> AQUI-S<sup>®</sup>20E) was calculated using the following equation:

$$A = [(B * C)/10^3]/10\% \text{ active eugenol}$$

$$A = \text{AQUI-S}^{\text{®}}20\text{E (g)}$$

$$B = \text{Volume of stock solution (L)}$$

$$C = \text{Target eugenol concentration (mg L}^{-1}\text{)}$$

#### Respirometry trials

AQUI-S<sup>®</sup>20E was weighed directly into a 50-mL beaker (Mettler Toledo Model AB265-S/FACT balance, Columbus, OH, USA). All stock solutions were prepared by adding the known mass of AQUI-S<sup>®</sup>20E to 10 L of water from the RAS tank followed by hand mixing for 1 min. Stock solutions of 0, 10, 20 and 30 mg L<sup>-1</sup> eugenol were individually prepared. A handheld pH meter (YSI Model 63; YSI Inc., Yellow Springs, OH, USA) was used to measure pH of the stock solution.

Stock solutions were used to fill the respirometry chamber, which was the experimental unit for the study. Blown air was supplied directly into the chamber using an air pump (Penn-Plax

Silent-AirX4<sup>™</sup>; Pentair Ltd., Minneapolis, MN, USA) with a terminal air diffuser. Fish were group weighed into different loading densities (Ohaus Explorer<sup>®</sup> Model EX4202 balance; Ohaus Corp., Parsippany, NJ, USA). Air was bubbled into the respirometry chamber until dissolved oxygen readings stabilized near 100% saturation. Once stabilized, the air diffuser was removed and fish were added to the chamber. The rubber stopper containing the temperature and dissolved oxygen probes (YSI Model 5350; Xylem Inc., Yellow Springs, OH, USA) was placed in the flask to seal the water from contact with the atmosphere and it was ensured that the flask was void of any air bubbles, which would potentially interfere with dissolved oxygen measurements. The flask, containing a 2.5 cm magnetic stir bar, was placed on top of a stir plate (Thermolyne Cimarec<sup>®</sup>2 Model S46725; Thermo Scientific, Lenexa, KS, USA) which was set to a slow, steady spin. Probes were connected to a YSI Model 5300 Biological Oxygen Monitor (Xylem Inc.) and a HOBO<sup>®</sup> 4-Channel External data logger (ONSET Inc., Bourne, MA, USA). The data logger recorded temperature and per cent saturation outputs from the oxygen monitor. Due to the higher metabolic rates of Nile tilapia, oxygen readings were recorded every 10 s for Nile tilapia and every 15 s for yellow perch. Fish entered the chamber at 100% saturation and remained in the chamber until dissolved oxygen dropped to ~50–60% saturation, which ranged from 2 to 12 min by treatment.

Wet weight (g) and TL (cm) were recorded for all fish. Wet weights for each treatment group were added together for use in mass-specific metabolic rate and loading density calculations. Survival was not assessed post exposure, however all fish were alive when the final wet weight and TL were measured.

#### Metabolic rate calculation

Mass-specific metabolic rates were calculated for each species of fish and for each combination of loading density and eugenol concentration (Cech 1990). Per cent saturation of oxygen was converted to mg L<sup>-1</sup> dissolved oxygen prior to calculating metabolic rates (USGS 2011). Calculations were conducted as follows:

$$MR = [(DO_{\text{initial}} - DO_{\text{final}}) \times V] \div (T \times M)$$

$$MR = \text{metabolic rate (mg O}_2\text{ kg}^{-1}\text{ h}^{-1}\text{)}$$

$$DO_{\text{initial}} = \text{dissolved oxygen concentration at an early point in experiment (mg L}^{-1}\text{)}$$

$DO_{\text{final}}$  = dissolved oxygen concentration at a late point in the experiment ( $\text{mg L}^{-1}$ )

V = volume of chamber (L)

T = time between initial and final (h)

M = mass of fish (kg)

### Statistical analyses

Data in tables were reported as mean  $\pm$  SD. Data presented graphically were expressed as mean  $\pm$  SEM. Homogeneity of variances was tested using Bartlett's test. Normality of data was tested using the Shapiro–Wilk test. A log-transformation was used to normalize data when the assumption of normality was invalidated. A one-way ANOVA and Tukey's HSD *post hoc* test were used to test for significant differences in metabolic rates for each loading density and eugenol concentration. In cases where transformations did not normalize the data, the non-parametric Kruskal–Wallis test was used to test significance. Analyses were performed using R (R Core Team 2013; 64-bit, version 2.15.3) with the R Commander interface (Fox 2005); significance was declared when  $P < 0.05$ .

### Results

The mean wet weight, TL and water quality parameters (temperature and pH) are summarized in Table 1. Fish weights from each treatment group were used to calculate the loading densities of each replicate (Table 2).

#### Yellow perch – 60, 120 and 240 $\text{g L}^{-1}$ loading densities

The three loading densities were tested independently at four eugenol concentrations (Fig. 1). Significant differences in metabolic rates were detected

**Table 1** Mean ( $\pm$ SD) total length (cm), wet weight (g) and water quality parameters (temperature and pH). Yellow perch had only one pH measurement therefore no variance estimator was reported

	Total length (cm)	Wet weight (g)	Temperature ( $^{\circ}\text{C}$ )	pH
Yellow Perch	10.1 (1.3)	9.74 (4.5)	16.2 (0.7)	7.93
Nile tilapia	11.4 (1.3)	25.91 (7.6)	21.5 (0.7)	7.95 (0.2)

for yellow perch exposed to 0, 10, 20 and 30  $\text{mg L}^{-1}$  eugenol at each of the loading densities (60  $\text{g L}^{-1}$ :  $F_{3,8} = 11.44$ ,  $P < 0.01$ ; 120  $\text{g L}^{-1}$ :  $F_{3,8} = 16.22$ ,  $P < 0.01$ ; and 240  $\text{g L}^{-1}$ :  $F_{3,8} = 14.93$ ,  $P < 0.01$ ). Yellow perch exposed to 30  $\text{mg L}^{-1}$  eugenol had significantly lower metabolic rates than the yellow perch exposed to 0 and 10  $\text{mg L}^{-1}$  eugenol at the 60  $\text{g L}^{-1}$  loading density ( $P < 0.05$ ). Yellow perch exposed to 20  $\text{mg L}^{-1}$  eugenol at the 60  $\text{g L}^{-1}$  loading density showed lower mean metabolic rates than the 0 and 10  $\text{mg L}^{-1}$  eugenol exposures, but the difference was not statistically significant. Metabolic rates of yellow perch at the 120  $\text{g L}^{-1}$  loading density were significantly lower for the 0, 20 and 30  $\text{mg L}^{-1}$  eugenol exposures relative to the 10  $\text{mg L}^{-1}$  exposure ( $P < 0.05$ ). The 240  $\text{g L}^{-1}$  loading density had significantly lower metabolic rates when exposed to 30  $\text{mg L}^{-1}$  eugenol relative to yellow perch exposed to 0, 10 and 20  $\text{mg L}^{-1}$  eugenol ( $P < 0.05$ ). Overall, yellow perch exposed to 20 and 30  $\text{mg L}^{-1}$  eugenol had lower mean metabolic rates than yellow perch exposed to 0 and 10  $\text{mg L}^{-1}$  eugenol at all three loading densities.

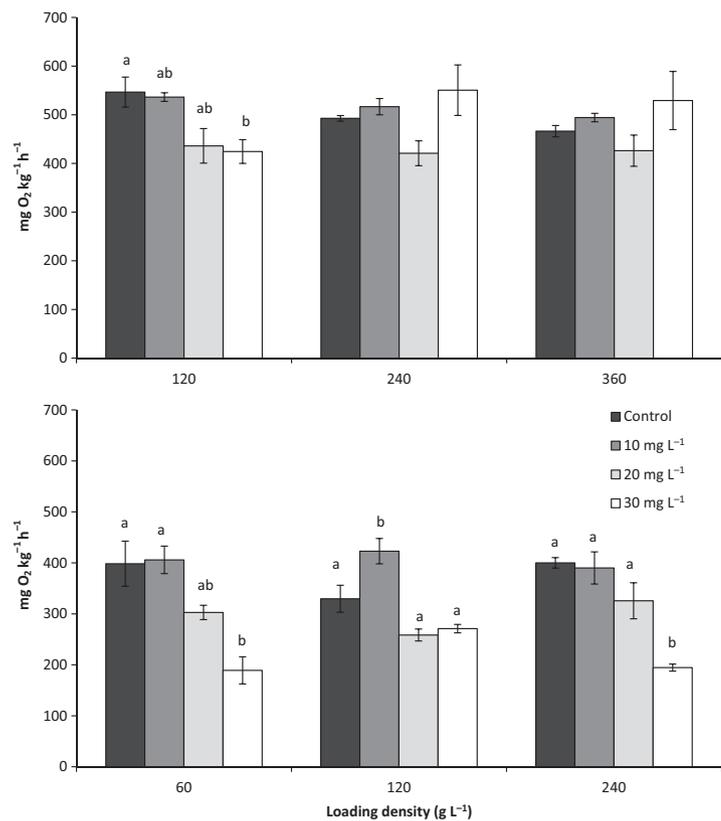
An additional comparison was conducted to determine the effect of loading density on metabolic rate at each eugenol concentration (Fig. 2). Significant differences among loading densities were detected for yellow perch exposed to 30  $\text{mg L}^{-1}$  eugenol ( $F_{2,6} = 7.613$ ,  $P < 0.05$ ). Yellow perch exposed to 30  $\text{mg L}^{-1}$  eugenol at the 120  $\text{g L}^{-1}$  loading density had significantly higher metabolic rates than yellow perch exposed to 30  $\text{mg L}^{-1}$  eugenol at the 60 and 240  $\text{g L}^{-1}$  loading densities ( $P < 0.05$ ). No significant differences in metabolic rates were detected for yellow perch exposed to 0, 10 and 20  $\text{mg L}^{-1}$  eugenol.

#### Nile tilapia – 120, 240 and 360 $\text{g L}^{-1}$ loading densities

The metabolic rates for Nile tilapia exposed to 0, 10, 20 and 30  $\text{mg L}^{-1}$  eugenol at the three

**Table 2** Loading densities ( $\pm$ SD) calculated from wet weight (g) of fish and respirometry chamber volume (L)

	Loading density ( $\text{g L}^{-1}$ )			
Yellow Perch	22.2 (2.7)	59.5 (2.3)	121.9 (4.3)	243.4 (6.9)
Nile tilapia	39.0 (8.7)	116.2 (7.0)	215.6 (10.2)	309.6 (13.7)



**Figure 1** Mean mass-specific metabolic rates [ $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  ( $\pm$  SEM bars)] for Nile tilapia (top) and yellow perch (bottom) at three high loading densities exposed to 0, 10, 20 and 30  $\text{mg L}^{-1}$  eugenol. Treatments with different letters are significantly different from each other ( $P < 0.05$ ).

loading densities are shown in Fig. 1. Metabolic rates for Nile tilapia exposed to 30  $\text{mg L}^{-1}$  eugenol at the 120  $\text{g L}^{-1}$  loading density were significantly lower than the metabolic rates of Nile tilapia exposed to 0  $\text{mg L}^{-1}$  eugenol at the 120  $\text{g L}^{-1}$  density ( $F_{3,8} = 5.764$ ,  $P < 0.05$ ). No other significant differences in metabolic rates were observed at the 240 or 360  $\text{g L}^{-1}$  loading densities when exposed to all four concentrations of eugenol.

Metabolic rates for Nile tilapia were compared at each eugenol concentration (Fig. 2). No significant differences in metabolic rates were detected between the three loading densities at 0, 10, 20 and 30  $\text{mg L}^{-1}$  eugenol.

#### Yellow perch – lowest density

Yellow perch exposed to eugenol at the two fish loading density (Fig. 3) resulted in significant differences in metabolic rates ( $F_{3,8} = 7.743$ ,  $P < 0.01$ ). The metabolic rate for yellow perch exposed to 30  $\text{mg L}^{-1}$  eugenol was significantly lower than the 0 and 10  $\text{mg L}^{-1}$  eugenol treatments ( $P < 0.05$ ). The 20  $\text{mg L}^{-1}$  eugenol treat-

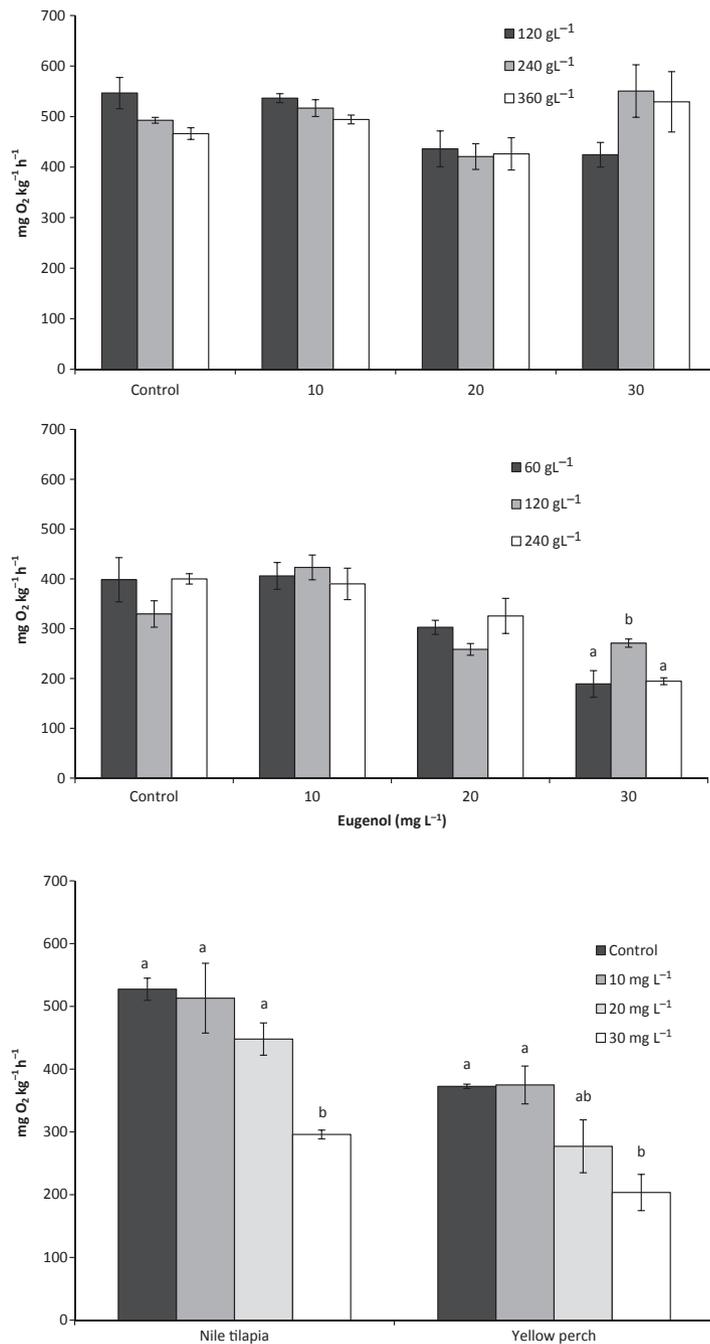
ment resulted in a decreased mean metabolic rate, but the decrease was not significantly lower than the 0 and 10  $\text{mg L}^{-1}$  eugenol exposures.

#### Nile tilapia – lowest density

Significantly reduced metabolic rates ( $F_{3,8} = 10.88$ ,  $P < 0.01$ ) were observed for Nile tilapia exposed to 30  $\text{mg L}^{-1}$  relative to 0, 20 and 20  $\text{mg L}^{-1}$  eugenol exposed Nile tilapia ( $P < 0.05$ ). The 20  $\text{mg L}^{-1}$  eugenol exposure resulted in a decreased metabolic rate relative to the 0 and 10  $\text{mg L}^{-1}$  eugenol exposures, but the decrease was not statistically significant.

#### Discussion

Respirometry experiments are important indicators to understand how fish respond to changes in their environment (Cech 1990). During events such as live transport, fish are subjected to a confined environment for potentially long durations (>1 h) at loading densities greater than typically encountered in most rearing systems (Ross & Ross 2008). The high loading densities of live transport



**Figure 2** Mean mass-specific metabolic rates [mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> (± SEM bars)] for Nile tilapia (top) and yellow perch (bottom) exposed to 0, 10, 20 and 30 mg L<sup>-1</sup> eugenol at three high loading densities. Treatments with different letters are significantly different from each other (*P* < 0.05).

**Figure 3** Mean mass-specific metabolic rates [mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> (± SEM bars)] for Nile tilapia and yellow perch at the lowest loading density when exposed to 0, 10, 20 and 30 mg L<sup>-1</sup> eugenol. No interspecies rate comparison was analysed. Treatments with different letters are significantly different from each other (*P* < 0.05).

expose fish to inadvertent stressors (Harmon 2009; Iverson, Eliassen & Finstad 2009) that can negatively impact post-transport survival (Kaiser & Vine 1998; Pearson, Small, Beecham, Sink, LaBarre & Minchew 2009). Fish transporters would benefit, both in efficiency and economically, from safely hauling fish at higher loading densities than are currently used in the industry without compromising fish welfare. Due to the gap of informa-

tion on eugenol sedation, the results of this study were compared with studies conducted using clove oil where eugenol is the primary active ingredient (70–90% by weight, Ross & Ross 2008).

### Yellow perch

Sufficient dissolved oxygen is a primary determinant in the success of live transport and dissolved

oxygen levels are generally inversely related to fish biomass (Piper, McElwain, Orme, McCraren, Fowler & Leonard 1982). This inverse relationship led Cai and Summerfelt (1992) to hypothesize that oxygen consumption may be the limiting factor for increasing fish biomass within tanks. Hence, this study exposed yellow perch to eugenol at the typical loading density of 60 g L<sup>-1</sup> and at elevated densities of 120 and 240 g L<sup>-1</sup> (Carmichael, Tomasso & Schwedler 2001).

Results of this study indicate that metabolic rates vary for yellow perch at each loading density when exposed to a range of eugenol concentrations. In general, as eugenol concentration increased, metabolic rate of yellow perch decreased. Decreased oxygen consumption has also been documented with increased concentrations of sedatives 2-phenoxyethanol (Guo, Teo & Chen 1995), isoeugenol (Forgan & Forster 2010) and clove oil (Hoskonen & Pirhonen 2004). However, this is the first report of a dose-dependent change in the metabolic rate of high densities of freshwater fish.

Eugenol exposure at 10 mg L<sup>-1</sup> did not significantly decrease metabolic rates relative to the control, conversely a higher metabolic rate was observed in eugenol exposed fish than the control at the 120 g L<sup>-1</sup> loading density. Results of several previous studies suggested that concentrations less than 10 mg L<sup>-1</sup> were effective to reduce the metabolic rate of fish. For example, 5–8.5 mg L<sup>-1</sup> clove oil effectively reduced oxygen demand in largemouth bass *Micropterus salmoides* most likely because cardiovascular output was reduced (Cooke *et al.* 2004). The cortisol stress response of matrinxa *Brycon cephalus* was reduced with 5 mg L<sup>-1</sup> clove oil when subjected to live transport, which would also decrease oxygen demand (Inoue, Afonso, Iwama & Moraes 2005). In addition, Iverson *et al.* (2009) measured the stress response of Atlantic salmon *Salmo salar* smolts exposed to clove oil during transport to sea and found that clove oil as low as 4.0 mg L<sup>-1</sup> was successful in reducing mortality. Results from this study suggest that a higher eugenol concentration (i.e.  $\geq 20$  mg L<sup>-1</sup> eugenol) is needed to induce a reduction in metabolic rates for yellow perch at elevated loading densities. In addition, to consistently decrease metabolic rates, yellow perch should be exposed to 30 mg L<sup>-1</sup> eugenol for loading densities up to 240 g L<sup>-1</sup>. The lower sedative concentrations from the previous studies can potentially

be attributed to their: (1) lower loading densities, (2) different physiological responses monitored, (3) interspecies differences, (4) additional active constituents of clove oil and (5) varying temperatures.

The lowest loading density ( $22.2 \pm 2.7$  g L<sup>-1</sup>) was much lower than typical transport density in production aquaculture (Carmichael *et al.* 2001). The significant reduction in metabolic rates when exposed to 30 mg L<sup>-1</sup> eugenol is important for understanding general sedation respiratory responses and aiding in low density transport (e.g. short duration transports <1 h, research purposes and intra-hatchery operations). The similarity between reduced metabolic rates at the elevated loading densities and this light loading density also suggested that the eugenol concentration and not the loading density alter the metabolic rates of yellow perch exposed to eugenol.

#### Nile tilapia

Nile tilapia at loading densities higher than currently recommended (i.e. 240 and 360 g L<sup>-1</sup>) showed no significant differences in metabolic rates when exposed to eugenol. Deriggi *et al.* (2006) observed no net ion imbalance in juvenile Nile tilapia exposed to 50 mg L<sup>-1</sup> eugenol for 10 min, but they hypothesized that oxygen debt may have occurred during sedation because of a suppressed metabolic rate. By contrast, eugenol (10–30 mg L<sup>-1</sup>) at 240 and 360 g L<sup>-1</sup> loading densities may not induce the same physiological response as observed by Deriggi *et al.* (2006) due to the lack of differences in metabolic rates.

Simoes, Lombardi, Gomide and Gomes (2011) exposed juvenile Nile tilapia to a range of clove oil concentrations in plastic bags at a loading density of  $\sim 22$  g L<sup>-1</sup> and found significant effluxes in ions and increased mortality for Nile tilapia at the highest concentration tested (18 mg L<sup>-1</sup> clove oil). While those results differ from our findings and those of Deriggi *et al.* (2006), the fish tested by Simoes *et al.* (2011) were much smaller ( $0.43 \pm 0.11$  g) and were sedated for a substantially longer duration (24 h). Physiological changes resulting from the effects of sedation have been found to be more pronounced in early life stage fish (Hoskonen & Pirhonen 2004).

Significantly lower metabolic rates were observed for Nile tilapia exposed to 30 mg L<sup>-1</sup> eugenol relative to controls (0 mg L<sup>-1</sup> eugenol) at the

120 g L<sup>-1</sup> loading density, a loading density that is commonly used in Nile tilapia transport. Nile tilapia held at this typical density may benefit from sedation using 30 mg L<sup>-1</sup> eugenol due to the reduction in metabolic rates (Carmichael *et al.* 2001).

Nile tilapia exposed to eugenol at the lowest loading density (39.0 ± 8.7 g L<sup>-1</sup>) showed significantly lower metabolic rates, when exposed to 30 mg L<sup>-1</sup> eugenol relative to all other eugenol concentrations. Although no significant differences in metabolic rates were found at the 240 and 360 g L<sup>-1</sup> loading densities for Nile tilapia exposed to 30 mg L<sup>-1</sup> eugenol, the significantly lower metabolic rates found at the 39 and 120 g L<sup>-1</sup> loading densities suggest that eugenol may be more effective at reducing metabolic rates at lower loading densities (e.g. general handling and sedation).

## Conclusion

Higher concentrations of eugenol than previously reported may be required to reduce metabolic rates when fish are held at high loading densities. Yellow perch at loading densities up to 240 g L<sup>-1</sup> had reduced metabolic rates when exposed to 20 and 30 mg L<sup>-1</sup> eugenol relative to the control. Exposure to 30 mg L<sup>-1</sup> eugenol also led to variable metabolic rates in yellow perch. Nile tilapia exposed to 30 mg L<sup>-1</sup> eugenol at a loading density of 120 g L<sup>-1</sup> had reduced metabolic rates relative to the control. Loading densities greater than 120 g L<sup>-1</sup> did not have reduced metabolic rates when exposed to eugenol. The results of this research suggest that eugenol can lower metabolic rates of different species held at high loading densities. Specifically, exposure of yellow perch to eugenol may result in reduced metabolic rates at current or increased loading densities while the effectiveness of eugenol to reduce metabolic rates of Nile tilapia appears to be more effective at lower loading densities.

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