Short communication

Confirmation that pulse and continuous peracetic acid administration does not disrupt the acute stress response in rainbow trout

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1. Introduction

Peracetic acid (PAA) has proven to be an efficient antimicrobial agent for aquaculture purposes (Meinelt et al., 2015; Liu et al., 2017a). In addition to its demonstrated effectiveness against fish pathogens (Farmer et al., 2013; Jussila et al., 2011; Smail et al., 2004; Lilley and Inglis, 1997), its degradation time and kinetics make it a good eco-friendly alternative to other disinfectants of common use in aquaculture such as formaldehyde, iodophors, phenolic compounds, chlorine or quaternary ammonium compounds (Danner and Merrill, 2005; Pedersen et al., 2009, 2013; Lahnsteiner and Kletzl, 2016; Liu et al., 2017a). Peracetic acid has been demonstrated to be acutely toxic to the following typical fish pathogens in vitro. Toxic concentrations of PAA were found to be < 0.3 mg L⁻¹ against Ichthyophthirius multifiliis theronts, 0.8 mg L⁻¹ against I. multifiliis tomonts, 1 mg L⁻¹ against Flavobacterium columnare and 4 mg L⁻¹ against Saprolegnia parasitica (see Meinelt et al., 2007, 2009; Straus and Meinelt, 2009; Marchand et al., 2012). Recent research has shown that fish are able to tolerate PAA at low concentrations. The 24-h no observed effect concentration (NOEC) for channel catfish (Ictalurus punctatus) yolk-sac fry was 2.2 mg L⁻¹ PAA and 1.3 mg L⁻¹ PAA for swim-up fry (Straus et al., 2012). The 24-h NOEC was 1.9–5.8 mg L⁻¹ PAA for a range of juvenile fish (Straus et al., in press). Several attempts of treating pathogens with PAA in the presence of fish were successful (Rintamaki-Kinnunen et al., 2005; Sudová et al., 2010; Jussila et al., 2011). To avoid the recurrence of pathogens, it is however necessary to use continuous or repeated exposures to PAA. In these cases, a welfare issue may emerge because the fish may suffer from chronic stress induced by the repeated exposures to PAA.

The stress response in common carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) was recently examined when fish were exposed to repeated applications of PAA; this was done in an attempt to identify welfare-related issues regarding the use of PAA in aquaculture facilities (Liu et al., 2017a,b). In these studies, exposed fish exhibited an increase in the levels of plasma cortisol (the most common stress marker in fish) after the initial exposure to PAA, which indicated that the exposure was stressful. After repeated exposures to PAA, the cortisol response of the fish was lower, suggesting that the fish might have become habituated to the exposure, which would support the use of
PAA as a welfare-friendly antimicrobial agent. However, lower levels of released cortisol are not necessarily the result of a process of habituation (Cyr and Romero, 2009). Alternative explanations to habituation for a cortisol response of smaller magnitude could be related to desensitization or exhaustion of the physiological stress response without habituation, or to potential PAA-induced alterations of the normal functioning of the hypothalamus-pituitary-interrenal (HPI) axis.

The present study determined whether these alternative explanations could be excluded, thus confirming that the fish are truly able to habituate to PAA exposure. According to our hypothesis, fish that are apparently habituated to PAA would be able to mount a normal physiological stress response (evaluated by measuring plasma cortisol, plasma glucose, plasma lactate and brain serotonergic activity) upon exposure to a different stressor.

2. Materials and methods

2.1. Fish, experimental design and sampling

Rainbow trout utilized during the study of Liu et al. (2017b) were used; their study evaluated how different types of PAA applications affected fish performance and system water quality in a flow-through aquaculture system. The applications were either repeated single dose (Pulse) or continuous via a peristaltic pump (Continuous). In brief, the following treatments were applied (in triplicate) to 180 L tanks containing 18 juvenile rainbow trout each: Control (no PAA exposure), Pulse (1 mg L\(^{-1}\) PAA, twice a week) and Continuous (0.2 mg L\(^{-1}\) PAA in the water inflow). This protocol was maintained for 6 weeks, after which the exposure experiment was completed. At that time, the average mass of the fish was 190.6 g (SD = 29.9 g) with no differences among treatment groups (Liu et al., 2017b).
The same experimental treatment groups and conditions were maintained for the present study; 48 h after the end of the exposure experiment described above, the fish were further exposed to a stress challenge of being pursued with a dipnet (chasing stress) for 1 min to evaluate the performance of the neuroendocrine stress pathways. The day of the experiment, 2 fish were quickly netted from one of the tanks and sampled as stress controls (time 0 treatment group). The net was then used to chase the remaining fish in the tank for 1 min. This procedure was repeated with the rest of the tanks. Therefore, 6 fish in total (2 per tank) were sampled as controls, for each of the treatments. At 1 h, 2 h, and 4 h after this stress, 8 fish from each treatment group were quickly netted and sampled. To minimize netting-induced stress, a single tank per treatment was dedicated exclusively to a particular sampling time. The fish were not fed for the 48 h prior to the experiment.

The sample procedure was as follows: fish were anesthetized in a 200 mg L\(^{-1}\) benzocaine solution; blood was collected from the caudal peduncle using 1-mL ammonium-heparinized syringes; the fish was then decapitated and the telencephalon was immediately dissected out, frozen rapidly in dry ice and later stored at \(-80^\circ\)C. The blood samples of each batch of fish were immediately centrifuged (6000 \(\times\) g, 4 \(^\circ\)C, 10 min) and the plasma was collected and stored at \(-80^\circ\)C for subsequent analyses of cortisol, glucose and lactate.

### 2.2. Biochemical analyses

Plasma cortisol was measured by means of a commercial ELISA kit (product #402710, Neogen Europe, Ayrshire, Scotland, UK), following the manufacturer’s instructions. Plasma glucose and plasma lactate were analyzed with colorimetric kits from Sigma (#MAK013, #MAK064, St. Louis, MO, USA).

The levels of serotonin (5-HT) and its main oxidative metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the telencephalon were analyzed using high performance liquid chromatography with electrochemical detection (HPLC-EC) as described by Gesto et al. (2017).

### 2.3. Data analysis

The differences in the response dynamics of the different stress markers among the three experimental treatment groups were assessed by two-way ANOVA using treatment group (Control, Pulse or Continuous) and time post-stress (0 h, 1 h, 2 h or 4 h) as factors. The ANOVA was followed by Holm-Sidak post-hoc tests to identify significant differences among treatments and among time groups. SigmaPlot version 12.5 (Systat Software Inc., San Jose, CA, USA) was used for all statistical analyses and the significance level was set at \(P \leq 0.05\).

### 3. Results and discussion

There were no differences in the plasma cortisol response to the chasing stressor (pursuit with a net) among the different treatment groups (Fig. 1 and Table 1). An increase in cortisol levels was observed at 1 h and a progressive recovery was evident at 2 h and 4 h after the chasing protocol. The cortisol response dynamics were consistent with other studies on chasing stress in rainbow trout (Gesto et al., 2013, 2015). The magnitude of the cortisol increase in the present study was smaller than in these other studies, most likely due to the limited duration of the stress protocol which was of only 1 min for the present study versus 3 min (Gesto et al., 2015) or 5 min (Gesto et al., 2013).

Plasma glucose also increased after the acute stress (Fig. 1). Plasma glucose levels usually increase upon stress exposure due to the action of cortisol and catecholamines (Wendelaar Bonga, 1997). The glucose increase is directed to provide the animal with energy to help it overcome the threat of the stressor (Wendelaar Bonga, 1997). Although the plasma glucose increase in the Pulse treatment group took longer to occur than in the Control and Continuous treatment groups, the ANOVA demonstrated no treatment-induced differences (Table 1). The dynamics of the glucose response was consistent to other studies with the same species and stressor type (Gesto et al., 2013, 2015). Plasma glucose levels did not recover within the time frame of the experiment. This is consistent with the typical dynamics of post-chasing stress glucose response in rainbow trout (Ings et al., 2012; Gesto et al., 2013), since it can take 8 h or more for the fish to recover pre-stress glucose levels.

Plasma lactate usually increases on exposure to different stressors (Milligan and Girard, 1993; Vijayan et al., 1997). This occurs as a result of a stress-induced increase in activity, with a corresponding higher degree of anaerobic glycolysis in the muscle (Van Ham et al., 2003; Iwama et al., 2006). We observed that the lactate response to stress showed the same trend than that of cortisol (Fig. 1). Plasma lactate increased 1 h after the stress and sequentially recovered to levels similar to those of the pre-stress state. At 2 h post-stress, lactate in all treatment groups was not different from the pre-stress levels. The magnitude of the increases and the dynamics of the lactate response (similar among all treatment groups) were analogous to the dynamics observed by Ings et al. (2012) and Gesto et al. (2013, 2015).

Habitation is a simple, nonassociative learning process by which animals reduce the magnitude of their response to a potentially deleterious stimulus after repeated exposures when the stimulus is recognized to be harmless (Grissom and Bhatnagar, 2009). Animals can generally habituate to stressors of low intensity (McCarty, 2016). A decrease in the cortisol response to PAA administration was observed after repeated exposures in rainbow trout and common carp (Liu et al., 2017a,b). It was suggested that the decrease was the result of habituation of the fish to mild stress generated by PAA administration. A reduction in the cortisol response to stress stimuli could, however, be due to different reasons and does not necessarily reflect a process of habituation. For example, the physiological stress responses can become desensitized or exhausted after repeated or chronic exposure to stress (Cyr and Romero, 2009; McKenzie et al., 2012; Barton et al., 2005; Madaro et al., 2015; Moltesen et al., 2016), even when the animals have not habituated to the stressor. Furthermore, different aquatic pollutants are capable of disrupting the normal function of the neuroendocrine pathways involved in the vertebrate stress response, altering the normal synthesis and release of stress hormones such as catecholamines and corticosteroids (Gesto et al., 2008; Ings et al., 2012; Hontela et al., 1997). A potential effect of PAA on these pathways cannot be excluded.

Altogether, the assessed plasma stress markers showed that when exposed to a secondary acute stressor, rainbow trout from different treatment groups (Pulse and Continuous) were equally able to develop a typical physiological stress response as opposed to fish that were never exposed to PAA (Control treatment group). This strongly suggests that

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>Treatment group</th>
<th>Time (\times) treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cortisol</td>
<td>(&lt; 0.001)</td>
<td>0.853</td>
<td>0.256</td>
</tr>
<tr>
<td>Glucose</td>
<td>(&lt; 0.001)</td>
<td>0.127</td>
<td>0.105</td>
</tr>
<tr>
<td>Lactate</td>
<td>(&lt; 0.001)</td>
<td>0.800</td>
<td>0.976</td>
</tr>
<tr>
<td>Brain</td>
<td>0.631</td>
<td>0.210</td>
<td>0.841</td>
</tr>
<tr>
<td>Serotonin (5-HT)</td>
<td>(&lt; 0.001)</td>
<td>0.843</td>
<td>0.575</td>
</tr>
<tr>
<td>5-hydroxyindoleacetic acid (5-HIAA)</td>
<td>(&lt; 0.001)</td>
<td>0.096</td>
<td>0.539</td>
</tr>
<tr>
<td>% 5-HIAA/5-HT</td>
<td>(&lt; 0.001)</td>
<td>0.575</td>
<td>0.105</td>
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the International Centre for Research in Organic Food Systems (ICROFS, Denmark) and the Green Development and Demonstration Programme (GUDP) under the Danish Ministry of Food, Agriculture and Fisheries.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pulse</th>
<th>Continuous</th>
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<tbody>
<tr>
<td>5-hydroxyindoleacetic acid (ng g⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>122.77 ± 5.23*</td>
<td>143.63 ± 13.13</td>
<td>130.39 ± 6.88</td>
</tr>
<tr>
<td>1 h</td>
<td>171.47 ± 9.15*</td>
<td>153.31 ± 4.02</td>
<td>154.41 ± 11.38</td>
</tr>
<tr>
<td>2 h</td>
<td>159.73 ± 13.97*</td>
<td>160.36 ± 8.31</td>
<td>160.81 ± 5.83</td>
</tr>
<tr>
<td>4 h</td>
<td>136.87 ± 8.03*</td>
<td>128.27 ± 10.83</td>
<td>130.66 ± 5.62</td>
</tr>
</tbody>
</table>

Serotonin (ng g⁻¹) |
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</thead>
<tbody>
<tr>
<td>0 h</td>
<td>472.68 ± 31.51</td>
<td>544.51 ± 19.40</td>
<td>491.14 ± 40.36</td>
</tr>
<tr>
<td>1 h</td>
<td>468.41 ± 19.67</td>
<td>518.14 ± 18.35</td>
<td>520.65 ± 32.28</td>
</tr>
<tr>
<td>2 h</td>
<td>500.16 ± 39.12</td>
<td>528.16 ± 23.74</td>
<td>509.16 ± 22.72</td>
</tr>
<tr>
<td>4 h</td>
<td>483.52 ± 18.10</td>
<td>478.31 ± 43.26</td>
<td>488.53 ± 20.79</td>
</tr>
</tbody>
</table>

Values represent the average (and SEM) of n = 6 – 8 fish. Different letters represent statistically significant differences (P < 0.05) among time points for a given parameter and experimental group.

References


Frost, G.M., Skov, P.V., Jokumsen, A., 2017. Emergence time and skin melanin spot pattern on rainbow trout, Oncorhynchus mykiss, following treatment by the Technical University of Denmark, the Leibniz-Institute of Freshwater Ecology and Inland Fisheries or the U.S. Department of Agriculture. The USDA is an equal opportunity provider and employer.

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