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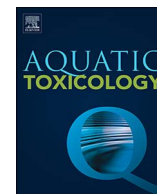
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# Physiological determinants of individual variation in sensitivity to an organophosphate pesticide in Nile tilapia *Oreochromis niloticus*

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## ABSTRACT

Individual variation in sub-lethal sensitivity to the organophosphate pesticide trichlorfon was investigated in Nile tilapia, using critical swimming speed ( $U_{crit}$ ) as an indicator. Tilapia exposed for 96 h to  $500 \mu\text{g l}^{-1}$  trichlorfon at  $26^\circ\text{C}$  (Tcfn group,  $n = 27$ ) showed a significant decline in mean  $U_{crit}$ , compared to their own control (pre-exposure) performance in clean water ( $-14.5 \pm 2.3\%$ , mean  $\pm$  SEM), but also compared to a Sham group ( $n = 10$ ) maintained for 96 h in clean water. Individuals varied in their relative sensitivity to the pesticide, with the decline in  $U_{crit}$  after exposure varying from 1 to 41%. The  $U_{crit}$  of the Tcfn group did not recover completely after 96 h in clean water, remaining  $9.4 \pm 3.2\%$  below their own control performance. The decline in performance was associated with a significant increase in net cost of aerobic swimming, of  $+28.4 \pm 6.5\%$  at a sustained speed of  $2 \text{ bodylengths s}^{-1}$ , which translated into a significant decline in swimming efficiency ( $E_{swim}$ ) of  $-17.6 \pm 4.0\%$  at that speed. Within the Tcfn group, individual  $E_{swim}$  was a strong positive determinant of individual  $U_{crit}$  across all trials, and a strong negative determinant of individual% decline in  $U_{crit}$  after pesticide exposure ( $P < 0.001$ , linear mixed effect models). Trichlorfon had no effects on standard metabolic rate or active metabolic rate (AMR) but, nonetheless, individual  $U_{crit}$  in all trials, and% decline in  $U_{crit}$  after exposure, were strongly associated with individual AMR (positively and negatively, respectively,  $P < 0.001$ ). Individual  $U_{crit}$  under control conditions was also a strong positive determinant of  $U_{crit}$  after trichlorfon exposure ( $P < 0.001$ ), but not of the% decline in  $U_{crit}$  performance. In conclusion, the OP pesticide impaired  $U_{crit}$  performance by reducing  $E_{swim}$  but individual tilapia varied widely in their relative sensitivity. Intrinsic individual physiology determined effects of the pesticide on performance and, in particular, good swimmers remained better swimmers after exposure.

## 1. Introduction

Organophosphate (OP) pesticides are used worldwide to control arthropod pests in agriculture and aquaculture, and often end up polluting waterways (Coelho et al., 2011; Guimar & es et al., 2007; Jordaan et al., 2013; Tierney et al., 2007; Varó et al., 2008). They are acetylcholinesterase (AChE) inhibitors with a wide range of toxic effects on animals besides target arthropods. In vertebrates they interfere irreversibly with cholinergic nerve function, at motor endplates and in the central nervous system. In fishes, sub-lethal doses of OP pesticides can

cause physiological and behavioural impairments of potential ecological significance (Coelho et al., 2011; Guimar & es et al., 2007; Jordaan et al., 2013; Tierney et al., 2007).

Exercise performance is a useful measure of sub-lethal toxic effects of pollutants on fishes, because activities such as foraging, escaping predators, protecting territory, and migration, all depend on swimming (Beamish, 1978; Cairns, 1966; McKenzie et al., 2007). OP pesticides impair exercise performance in fishes (Cripe et al., 1984; Peterson, 1974; Tierney et al., 2007) when measured as their critical swimming speed ( $U_{crit}$ ; Brett, 1964). In coho salmon *Oncorhynchus kisutch*, a

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decline in  $U_{crit}$  performance following exposure to chlorpyrifos was associated with reduced AChE activity in slow-twitch aerobic muscle. Vertebrate skeletal muscle requires AChE to function, so impaired  $U_{crit}$  performance was attributed to compromised neuromuscular coordination (Tierney et al., 2007). OPs are also reported to influence the metabolism and cardiorespiratory physiology of fishes, reducing metabolic rate, heart rate, ventilatory activity and spontaneous swimming activity (da Silva et al., 1993; De Aguiar et al., 2004; Gehrke, 1988; Tryfonos et al., 2009). In the Nile tilapia *Oreochromis niloticus*, trichlorfon decreased the ability to regulate aerobic metabolism in hypoxia, which was linked to an impaired capacity to hyperventilate (Thomaz et al., 2009). Thus, reduced exercise performance following OP exposure in fishes may reflect direct effects on swimming muscles but also impacts on respiratory metabolism and the ability of the cardiorespiratory system to meet the oxygen demands of activity.

Investigations in fish ecotoxicology typically focus upon understanding mean responses to pollutants in the population of interest, with less focus on how individuals might vary in their sensitivity (Claireaux et al., 2013; Kolok et al., 1998, 2002; Sloman, 2007). The study of individual variation in responses to pollutants can, however, provide insights into the ability of the population to survive exposure, and into the nature of the individuals that will then compose it (Brown et al., 2009; Calow and Forbes, 1998; Claireaux and Chabot, 2016; Kolok et al., 1998). Individuals in fish species show wide and repeatable variation in complex physiological traits of exercise performance and respiratory metabolism. This includes variation in  $U_{crit}$  performance (Claireaux et al., 2005; Kolok et al., 1998; Marras et al., 2010); standard metabolic rate (SMR, the basal metabolic rate of an ectotherm acclimated to a given temperature), and active metabolic rate (AMR, the highest achievable rate of oxygen uptake at the acclimation temperature) (Killen et al., 2012, 2011; Norin and Malte, 2011). Variation in these traits may influence an individual's sensitivity to sub-lethal effects of pollutants such as OP pesticides. This is significant because it has been argued that individual variation in these traits can be related to variation in life history traits such as growth rate, and in behavioural traits such as activity, boldness, sociability, or aggression (Biro and Stamps, 2010; Killen et al., 2016; Metcalfe et al., 2016). Therefore, sub-lethal exposure to contaminants could have direct or correlated selective effects, inducing shifts in the fundamental characteristics of impacted populations.

This study investigated negative effects of 96 h exposure to a sub-lethal concentration ( $500 \mu\text{g l}^{-1}$ ) of Neguvon®, a commercial formulation of trichlorfon, on  $U_{crit}$  performance in Nile tilapia (Thomaz et al., 2009). The Nile tilapia was introduced into Brazil in the 1950s and is now widespread and an important food fish. The exposure concentration is a standard prophylactic dose against ectoparasites in Brazilian tilapia farming, which is expected to have no long-term negative effects on their physiology and performance (Coelho et al., 2011; Guimarães and Calil, 2008; Thomaz et al., 2009). Reported concentrations of OP pesticides in surface waters are typically much lower (e.g. Leong et al., 2007; Sankararamkrishnan et al., 2005; Veiga et al., 2006) but there are major concerns about indiscriminate and unsupervised application of OP pesticides in crop treatments, livestock dips and pond aquaculture, whose transient effects on local waterways are not monitored (Chang et al., 2006; Peres and Moreira, 2007; Sturve et al., 2016; Veiga et al., 2006).

Individual variation in sensitivity to trichlorfon, and in the ability to recover  $U_{crit}$  performance after 96 h in uncontaminated water, was evaluated. Swimming respirometry was used to investigate proximate mechanisms of diminished  $U_{crit}$  performance, focussing on impairments of neuromuscular function, measured as swimming efficiency ( $E_{swim}$ ), and impacts on metabolism and respiratory performance, measured as SMR and AMR. The extent to which sensitivity to trichlorfon depended upon individual  $E_{swim}$ , SMR, AMR, and intrinsic  $U_{crit}$  performance, was then evaluated.

## 2. Methods

### 2.1. Animals

Juvenile *O. niloticus* of both sexes ( $n = 37$ ), aged about 5 months and with a mass of approximately 30 g, were provided by Piscicultura Poletini (pisciculturapoletini.blogspot.com) in Mogi Mirim (São Paulo state) and transported by road to the Department of Physiological Sciences, Federal University of São Carlos (São Carlos, SP). There they were maintained in a  $1.5 \text{ m}^3$  tank supplied with water at  $25 \pm 1^\circ\text{C}$  from a recirculating, biofiltered system, under a natural photoperiod, and provided with commercial feed daily at approximately 2% body mass  $\text{d}^{-1}$ , for 12 weeks, by which time they had a mass of approximately 90 g. Animals were then tagged (Passive Integrated Transponder) in the dorsal epaxial muscle under mild anaesthesia ( $0.1 \text{ g l}^{-1}$  benzocaine), for individual identification, after which they recovered in routine holding conditions for at least one week before use in the experiments. All experiments were performed at  $26 \pm 0.1^\circ\text{C}$ .

### 2.2. Exposure protocol

Individuals were submitted to three sequential  $U_{crit}$  swimming respirometry tests (see methods below). A first control test (Con) was performed on individuals held under routine conditions. Prior to the Con test, individuals were weighed to the nearest g and measured to the nearest mm, then placed in a separate  $1.5 \text{ m}^3$  tank in the biofiltered system and fasted for 24 h. After the Con test, the individuals were allowed at least 48 h recovery in their normal holding conditions, prior to further experimentation.

Individuals were then exposed for 96 h to either trichlorfon (Tcfn group,  $n = 27$ ) or to uncontaminated water (Sham group,  $n = 10$ ). For the Tcfn group, individual tilapia were placed in plastic tubs (volume 100 l) containing 60 l of biofiltered freshwater at 12:00. At 16:00, a solution of Neguvon® (Bayer Saude Animal, [www.bayersaudeanimal.com.br](http://www.bayersaudeanimal.com.br)) containing 97% trichlorfon and 3% neutral adjuvant, was added to the water to achieve a final concentration of  $500 \mu\text{g l}^{-1}$  trichlorfon (Thomaz et al., 2009) in the tub. At 24 h, 48 h and 72 h, 50% of the water was removed and replaced with fresh water containing trichlorfon at the appropriate dose. Water volumes were measured by gravimetry to minimise variation in conditions among the individual exposures. Water was aerated and mixed with an airstone and the tub was shielded with black plastic sheeting to avoid disturbance to the fish. The Sham group was treated identically but with no trichlorfon added to the water. At 96 h all individuals were submitted to an 'experimental' (Exp) swim test.

A third test was then performed after 96 h recovery (Rec) in uncontaminated water. That is, at fatigue from the Exp test, each tilapia was placed in a clean plastic tub, in 60 l of biofiltered water. 50% of the water was changed each 24 h after their placement into the tub, up to 96 h, when they were submitted to the Rec swim test.

### 2.3. Swimming performance and respirometry

Swimming respirometry was performed with a Steffensen-type swim-tunnel respirometer constructed of Plexiglas (volume  $13.4 \text{ l}$ ), designed to exercise fish in a non-turbulent water flow with a uniform velocity profile. The tunnel has been described in detail previously (McKenzie et al., 2007). Tilapia were transferred to the respirometer without air-exposure, by coaxing them into a plastic bag underwater, placing the bag into the water-filled swim channel of the tunnel, and gently releasing them (McKenzie et al., 2007). For the Exp tests, all animals (Tcfn and Sham groups) were first transferred from their exposure tub into a bucket, using the plastic bag method. The bag and bucket were then flushed with clean biofiltered water for 5 min, in a sink, after which the tilapia were again coaxed into the bag and transferred to the swim tunnel. The objective was to ensure that, for

individuals from the Tcfn group, there was no risk of contaminating the swim tunnel with pesticide. The swimming respirometer was provided with a flow of well-aerated uncontaminated water from the biofiltered system, and fish were allowed 4 h recovery, swimming at a current speed equivalent to 1 bodylength  $s^{-1}$  ( $BL s^{-1}$ ). At this speed they maintained position with pectoral sculling and gentle tailbeats (McKenzie et al., 2003). The anterior half of the swim chamber was covered with opaque black plastic sheeting, to avoid disturbing the fish, which could be observed from behind.

They were then exposed to stepwise increments in swimming speed every 30 min, of 1  $BL s^{-1}$  until 3  $BL$ , then at 0.5  $BL s^{-1}$  until they fatigued. All swimming speeds were corrected for the blocking effect of the fish (Bell and Terhune, 1970). Fatigue was unequivocal in the tilapia, they all swam vigorously until they collapsed against the rear screen of the swim tunnel and could not be persuaded to resume swimming by gentle manual encouragement (McKenzie et al., 2003). Current speed was then reduced to 1  $BL s^{-1}$  and the fishes removed, blotted dry and weighed to the nearest g. They were then immediately placed into the appropriate next step of the exposure protocol or, after the Rec test, killed by an overdose of anaesthesia (0.5 g  $l^{-1}$  benzocaine for ten min) followed by a blow to the head. The  $U_{crit}$  ( $BL s^{-1}$ ) was calculated as described previously (Brett, 1964), with an equation which adds the velocity of the most recently completed increment to the product of the incremental increase in velocity and the proportion of the final increment completed before fatigue.

Measurements of  $O_2$  uptake ( $M_{O_2}$ , in  $mmol kg^{-1} h^{-1}$ ) were made at each swimming speed by intermittent stopped-flow respirometry (Steffensen, 1989) over a 15 min cycle, providing two measures of  $M_{O_2}$  for each incremental swimming speed that was completed. Water oxygen concentration was recorded continuously using an optical oxygen probe and meter (Fibox. Pre-sens GmbH. [www.presens.de](http://www.presens.de)) and associated software (Pre-sens Oxyview). The  $M_{O_2}$  was then calculated considering the rate of decline in oxygen concentration, the water volume in the swim tunnel and the mass of the fish (McKenzie et al., 2007, 2003). Blank tests were run at the end of each swim test, to correct for the contribution of bacterial metabolism. This was never greater than 10% of  $M_{O_2}$  by the fish.

Oxygen uptake at each swimming speed was calculated as the average of the two respirometry cycles. Net metabolic cost of swimming (COS) was calculated at a sustained speed of 2  $BL s^{-1}$ , as the mean  $M_{O_2}$  at that speed minus the appropriate SMR (Beamish, 1978; McKenzie et al., 2003; see below for calculation of SMR). Swimming efficiency ( $E_{swim}$ ) was then derived at the speed of 2  $BL s^{-1}$ , in  $BL \mu mol O_2^{-1} kg^{-1}$ , by simply dividing the net cost of swimming by the speed in  $BL$  (i.e. 2). For each individual fish and swim test, a least-squares exponential regression was applied to the relationship between swimming speed and the  $M_{O_2}$  measures at each speed. Extrapolation back to the y-intercept, a notional swimming speed of zero, was employed to correct for the contribution to  $M_{O_2}$  of locomotor muscle activity (Brett, 1964; Fry, 1971). This was considered an estimate of SMR under the prevailing condition that, in the Con swim test, should have been an estimate of the individual's intrinsic SMR (Brett, 1964; Chabot et al., 2016; McKenzie et al., 2003). Active metabolic rate (AMR) was estimated as the highest single measure of  $M_{O_2}$  measured during the swim protocol (McKenzie et al., 2003; Norin and Clark, 2016), and individual AS calculated as AMR-SMR (Brett, 1964; McKenzie et al., 2003).

#### 2.4. Statistical analyses

A two-way ANOVA for repeated measures was used to compare Sham and Tcfn groups across the three swim tests, with one factor the Group, the repeated factor the Swim test and each fish as a subject. Linear mixed effect models (LMEs) were constructed to investigate proximate determinants of changes in  $U_{crit}$  performance in the Tcfn group. In one,  $U_{crit}$  was the dependent variable, considering all three swim tests (Con, Exp then Rec), with fish mass, fork length, swim test,

$E_{swim}$  at 2  $BL s^{-1}$ , SMR and AMR as explanatory fixed effects. In a second model, % decline in  $U_{crit}$  from the Con test was considered as a dependent variable for the Exp and Rec tests, with the same explanatory fixed effects. Further LME models were then performed to investigate whether  $U_{crit}$  performance after pesticide exposure was determined by intrinsic physiology of the individual, based upon their metabolism and performance in the Con swim test. In these, absolute  $U_{crit}$  in Exp and Rec tests, and % decline in  $U_{crit}$  from the Con test, were the dependent variables. Swim test (Exp or Rec), fish mass, forklength,  $E_{swim}$  at 2  $BL s^{-1}$ , Con SMR, Con and Con AMR were explanatory fixed effects.

As each fish was tested under multiple conditions, fish identity was a random effect in all LMEs, and swim test a repeated factor. Initial models included all relevant interactions, non-significant interactions were sequentially dropped and models rerun. Model assumptions of homogeneity, linearity, and normality of residuals were verified by inspection of residuals-fits plots. Statistics were performed with SigmaPlot ([www.systat.com](http://www.systat.com)) and SPSS Statistics v17.0 ([www.ibm.com/software/analytics/spss](http://www.ibm.com/software/analytics/spss)). Significance for all tests was  $\alpha = 0.05$ .

### 3. Results

#### 3.1. Effects of trichlorfon on swimming performance and metabolism

Complete swim test and respirometry data were collected for  $n = 10$  Sham and  $n = 27$  Tcfn (Table 1). Reporting of ANOVA results is limited to effects within each group, and comparisons of swim tests between groups. There was no significant change in any variable in the Sham group, across the three swim tests (Table 1, Fig. 1). In contrast, mean  $U_{crit}$  in the Tcfn group declined significantly from Con to Exp tests and then showed only a partial recovery, with mean Tcfn Rec  $U_{crit}$  being higher than Tcfn Exp but still significantly lower than Tcfn Con (Fig. 1). The mean Tcfn Exp  $U_{crit}$  was significantly lower than the Sham Exp  $U_{crit}$ , although Rec  $U_{crit}$  was not significantly different between the two groups (Fig. 1). Within the Tcfn group, the mean ( $\pm$  SE) percentage decline in the Exp test was  $14.1 \pm 2.4\%$  when compared to the Con test, but this varied from 0.7% to over 41% among the 27 individuals. For the Rec test, the mean decline from Con was  $9.3 \pm 3.2\%$  with much variation among individuals, from +28% (i.e. improved performance) to -40%.

In the Tcfn group, the decline in  $U_{crit}$  from the Con to the Exp and Rec tests was associated with significant changes in variables derived from their oxygen uptake at a speed of 2  $BL s^{-1}$  (Fig. 1). That is, COS at

**Table 1**

Mean ( $\pm$  SE) values for standard metabolic rate (SMR), active MR (AMR) and aerobic scope (AS) in Nile tilapia *Oreochromis niloticus*, during a control swim test (Con); a test after 96 h exposure (Exp) to either uncontaminated water (Sham group) or 500  $\mu g l^{-1}$  trichlorfon (Tcfn group), and then a final test after 96 h recovery (Rec) in uncontaminated water.

	Sham	Tcfn
n	10	27
Initial mass (g)	95 $\pm$ 4	99 $\pm$ 3
Forklength (mm)	180 $\pm$ 3	183 $\pm$ 3
Con test		
SMR	4.1 $\pm$ 0.5	4.1 $\pm$ 0.2
AMR	16.8 $\pm$ 1.3	15.7 $\pm$ 0.6
AS	12.6 $\pm$ 0.1	11.6 $\pm$ 0.6
Exp test		
SMR	4.1 $\pm$ 0.4	4.1 $\pm$ 0.2
AMR	18.2 $\pm$ 1.1	16.9 $\pm$ 0.7
AS	14.1 $\pm$ 1.1	12.8 $\pm$ 0.6
Rec test		
SMR	3.8 $\pm$ 0.4	4.4 $\pm$ 0.3
AMR	18.5 $\pm$ 1.1	16.7 $\pm$ 0.9
AS	14.8 $\pm$ 1.0	12.3 $\pm$ 0.9

SMR, AMR and AS are in  $mmol O_2 kg^{-1} h^{-1}$ .

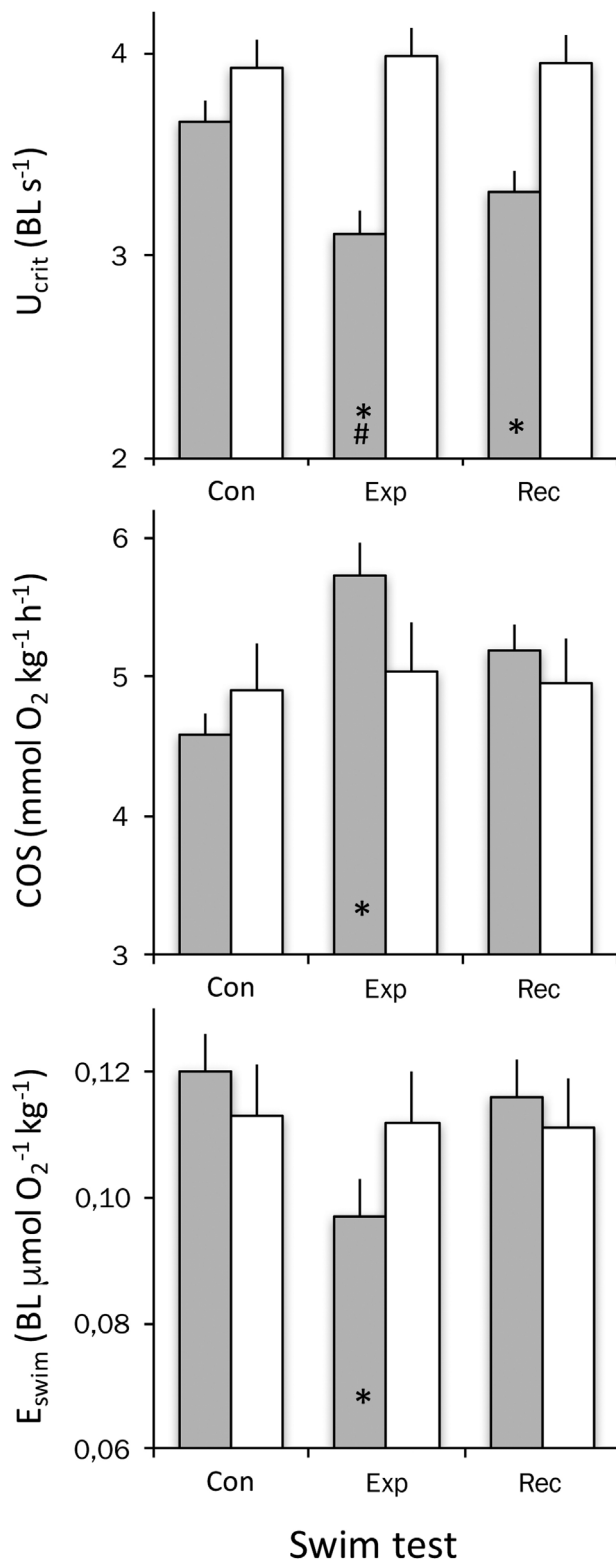


Fig. 1. Mean ( $\pm$  SE) critical swimming speed ( $U_{crit}$ ) and, for a sustained speed of 2 bodylengths s<sup>-1</sup>, net cost of swimming (COS) and swimming efficiency ( $E_{swim}$ ) in Nile tilapia *Oreochromis niloticus*, during a control swim test (Con); a test after 96 h exposure (Exp) to either 500 µg l<sup>-1</sup> trichlorfon (Tcfn group, grey bars) or uncontaminated water (Sham group, white bars) and then a final test after 96 h recovery (Rec) in uncontaminated water.  $n = 27$  for Tcfn, 10 for Sham. Asterisk indicates significant difference from Con test for that group, hash tag indicates significant difference between Tcfn and Sham group for that test ( $P < 0.05$  by two-way ANOVA for repeated measures).

Table 2

Results of a linear mixed effect model to investigate how critical swimming speed (in BL s<sup>-1</sup>) depended on various possible explanatory variables in  $n = 27$  Nile tilapia exposed to three sequential swim tests: a control swim test (Con); a test after 96 h exposure to 500 µg l<sup>-1</sup> trichlorfon (Exp), and then a final test after 96 h recovery in uncontaminated water. Estimates are for fixed effects of the three swim tests; log body mass (g); log standard metabolic rate (SMR, mmol O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>); log swimming efficiency at 2 BL s<sup>-1</sup> ( $E_{swim}$ , µmol O<sub>2</sub> BL<sup>-1</sup> kg<sup>-1</sup>), and log active metabolic rate (AMR, mmol O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>). See the text for further details. Significant effects are in bold.

Parameter	Estimate	SE	df	t	P
Intercept	6.07	1.48	36.4	4.09	< 0.001
Con swim test	0.21	0.10	30.8	2.20	0.035
Exp swim test	-0.12	0.09	29.8	-1.34	0.191
Log mass	-1.62	0.70	29.3	-2.35	0.026
Log $E_{swim}$	2.65	0.41	39.8	6.51	< 0.001
Log SMR	-0.48	0.27	50.8	-1.77	0.083
Log AMR	2.78	0.40	54.8	6.89	< 0.001

\* Parameter for REC test is redundant.

2 BL s<sup>-1</sup> increased significantly, by  $28.4 \pm 6.5\%$ , from Tcfn Con to Exp swim tests, which translated into a significant  $17.6 \pm 4.0\%$  decline in  $E_{swim}$  (Fig. 1). As a result, there was some indication that mean Tcfn Exp COS and  $E_{swim}$  were lower than their Sham Exp counterparts ( $P \approx 0.07$ ). The Tcfn Rec COS and  $E_{swim}$  were statistically similar to both Con and Exp swim tests, and were statistically similar to their Sham counterparts (Table 1, Fig. 1). There were no significant changes in mean SMR, AMR or AS across the three tests (Table 1).

### 3.2. Determinants of individual variation in sensitivity in the Tcfn group

Within the Tcfn group, there was an overall negative dependence of individual  $U_{crit}$  upon log body mass, with no interaction with swim trial (Table 2). Individual  $E_{swim}$  was a strong determinant of individual swimming performance under all circumstances. There was a strong overall positive dependence of  $U_{crit}$  upon log  $E_{swim}$  (Table 2), plus a strong negative dependence of individual% decline in  $U_{crit}$ , between the Con trial and the Exp or Rec trials, upon log  $E_{swim}$  in those trials (Table 3). There was weak evidence ( $P = 0.08$ ) of an overall negative effect of individual log SMR on  $U_{crit}$  (Table 2). There was also, however, a strong link between individual AMR and individual performance. That is, there was a strong overall positive dependence of individual  $U_{crit}$  upon log AMR (Table 2) and a strong negative dependence of individual% decline in  $U_{crit}$ , between the Con trial and the Exp or Rec trials, upon log AMR in those trial (Table 3).

The individuals'  $U_{crit}$  in the Con trial was a significant positive determinant of their  $U_{crit}$  in the Exp and Rec trials (Table 4). Linear regressions of  $U_{crit}$  in the Exp and Rec trials, against Con  $U_{crit}$ , showed significant correlations across trials (Fig. 2). Although linear regressions only capture a portion of the information considered in the statistical

Table 3

Results of a linear mixed effect model to investigate how the percentage decline in critical swimming speed, from a control swim test in uncontaminated water to a test swim after 96 h exposure to 500 µg l<sup>-1</sup> trichlorfon, and then a further test swim after 96 h recovery in uncontaminated water, depended upon various possible explanatory variables in  $n = 27$  Nile tilapia. Estimates are for fixed effects of the two swim tests; log body mass (g); log standard metabolic rate (SMR, mmol O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>); log swimming efficiency at 2 BL s<sup>-1</sup> ( $E_{swim}$ , µmol O<sub>2</sub> BL<sup>-1</sup> kg<sup>-1</sup>) and log active metabolic rate (AMR, mmol O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>). See the text for further details. Significant effects are in bold.

Parameter	Estimate	SE	df	t	P
Intercept	78.44	48.64	34.6	1.61	0.12
Swim test	3.99	2.67	26.4	1.50	0.15
Log mass	-24.30	21.61	32.0	-1.12	0.27
Log $E_{swim}$	-71.36	16.27	37.4	-4.39	< 0.001
Log SMR	11.28	9.78	34.5	1.15	0.26
Log AMR	-81.84	15.00	41.4	-5.46	< 0.001

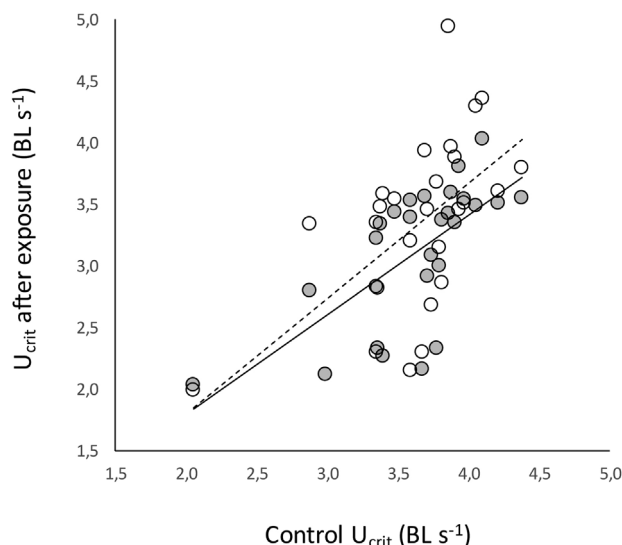
\*Parameter set to zero because redundant.



**Table 4**

Results of a linear mixed effect model to investigate whether critical swimming speed ( $U_{crit}$ , in  $BL\ s^{-1}$ ), measured in a swim test after 96 h exposure to  $500\ \mu g\ l^{-1}$  trichlorfon (Exp test) and then again in a test after 96 h recovery in uncontaminated water (Rec test), depended upon various traits measured under control conditions prior to pesticide exposure, in  $n = 27$  Nile tilapia. Estimates are for fixed effects of the two swim tests, then for control  $U_{crit}$ , log body mass (g), log standard metabolic rate (SMR,  $mmol\ O_2\ kg^{-1}\ h^{-1}$ ), log swimming efficiency at  $2\ BL\ s^{-1}$  ( $E_{swim}$ ,  $\mu mol\ O_2\ BL^{-1}\ kg^{-1}$ ) and log active metabolic rate (AMR,  $mmol\ O_2\ kg^{-1}\ h^{-1}$ ). See the text for further details. Significant effects are in bold.

Parameter	Estimate	SE	df	t	P
Intercept	2.39	2.66	21.0	0.90	0.30
Swim test	−0.21	0.12	26.0	−1.72	0.096
Log control mass	−1.59	0.98	21.0	−1.62	0.12
Control $U_{crit}$	0.92	0.22	21.0	4.22	< 0.001
Log control $E_{swim}$	−2.02	1.17	21.0	−1.72	0.099
Log control SMR	0.47	0.64	21.0	0.74	0.46
Log control AMR	−1.17	1.14	21.0	−1.03	0.31



**Fig. 2.** Least-squares linear regression of  $U_{crit}$  after 96 h exposure to  $500\ \mu g\ l^{-1}$  trichlorfon (grey dots – simple regression line,  $R^2 = 0.448$ ,  $P = 0.00015$ ), or after 96 h recovery in clean uncontaminated water (white dots, dashed regression line,  $R^2 = 0.351$ ,  $P = 0.00113$ ), as a function of  $U_{crit}$  measured prior to exposure, under control conditions in clean water.  $n = 27$ ,  $U_{crit}$  in bodylengths  $s^{-1}$ .

**Table 5**

Results of a linear mixed effect model to investigate whether the percentage decline in critical swimming speed ( $U_{crit}$ ), from a control swim test in uncontaminated water to a test after 96 h exposure to  $500\ \mu g\ l^{-1}$  trichlorfon (Exp test), and then a further test after 96 h recovery in uncontaminated water (Rec test), depended upon various traits measured under the control conditions, in  $n = 27$  Nile tilapia. Estimates are for fixed effects of swim test (Exp vs Rec) and control  $U_{crit}$  ( $BL\ s^{-1}$ ), log body mass (g), log standard metabolic rate (SMR,  $mmol\ O_2\ kg^{-1}\ h^{-1}$ ), log swimming efficiency at  $2\ BL\ s^{-1}$  ( $E_{swim}$ ,  $\mu mol\ O_2\ BL^{-1}\ kg^{-1}$ ) and log active metabolic rate (AMR,  $mmol\ O_2\ kg^{-1}\ h^{-1}$ ).

Parameter	Estimate	SE	df	t	P
Intercept	60.4	75.5	21.0	0.80	0.43
Swim test	5.6	3.3	26.0	1.67	0.11
Log control mass	44.7	27.9	21.0	1.60	0.12
Control $U_{crit}$	0.1	6.18	21.0	0.01	0.99
Log control $E_{swim}$	58.6	33.2	21.0	1.76	0.09
Log control SMR	−11.0	18.1	21.0	−0.60	0.55
Log control AMR	34.0	32.3	21.0	1.05	0.30

model, Fig. 2 visually demonstrates that, although  $U_{crit}$  performance varied quite widely among the individuals, their relative performance was consistent across contexts, especially for the Con to Exp trial, less so for the Con to Rec trial (Fig. 2). Control  $E_{swim}$ , SMR and AMR were not, however, determinants of  $U_{crit}$  after pollutant exposure, although the

effect of intrinsic  $E_{swim}$  approached significance (Table 4). In terms of the percentage decline in  $U_{crit}$  performance, from Con to Exp or Rec trials, this was independent of all aspects of intrinsic physiology (Table 5) although a dependence on Con  $E_{swim}$  approached significance.

#### 4. Discussion

The OP pesticide trichlorfon caused prolonged impairments to  $U_{crit}$  swimming performance in *O. niloticus*, but individuals varied widely in their relative sensitivity. The proximate mechanism underlying the decline in  $U_{crit}$  after pesticide exposure was a decline in swimming efficiency; trichlorfon had no effect on respiratory metabolism. Although an individual's  $E_{swim}$  was a direct determinant of their  $U_{crit}$  performance, individual AMR was also strongly linked to relative performance across all trials.  $U_{crit}$  measured under control conditions was a strong predictor of performance after pesticide exposure, showing that relative aerobic swimming performance was maintained across contexts.

##### 4.1. Effects of trichlorfon on swimming performance persisted for at least 96 h

The decline in  $U_{crit}$  in *O. niloticus* exposed to trichlorfon is similar to negative effects of OP pesticides on swimming performance of other fishes (Cripe et al., 1984; Peterson, 1974; Tierney et al., 2007). This is the first study to investigate the ability of fishes to recover  $U_{crit}$  after a sub-lethal OP exposure, revealing that the effects of the pesticide persisted for at least 96 h after return to uncontaminated water. Previous studies have found long-lasting effects of OP pesticides on activities of AChE activity in plasma and brain of tropical fishes at a similar temperature to this study (De Aguiar et al., 2004). The OPs are irreversible AChE inhibitors; cleavage of an OP by AChE leaves a phosphoryl group in the esteratic site, which is slow to be hydrolyzed (on the order of days) and can become covalently bound (Coelho et al., 2011).

This long-lasting effect is of clear ecological significance (De Aguiar et al., 2004). Although the trichlorfon concentration used in this study may seem relatively high, it has been proposed as a standard prophylactic dose against ectoparasites in fish culture that, presumably, is expected to have no long-term negative effects on their physiology and performance (Coelho et al., 2011; Guimarães and Calil, 2008; Thomaz et al., 2009). In many countries with major agriculture and aquaculture sectors, there are concerns that OP pesticides are used almost indiscriminately, with no specialised supervision or monitoring of their fate in local waterways (Chang et al., 2006; Coelho et al., 2011; De Aguiar et al., 2004; Guimarães et al., 2007; Linde-Arias et al., 2008; Peres and Moreira, 2007; Sturve et al., 2016; Veiga et al., 2006). In aqueous solution trichlorfon hydrolyses into the much more potent AChE inhibitor dichlorvos, in a manner that depends upon pH and temperature, but which can lead to more severe toxic effects (Tronczynski, 1990). There is a risk, therefore, of wild fish populations being exposed to transient pulses of OP pesticides due to runoff from field treatments, livestock dips or flow-through aquaculture ponds, especially during the rainy season (Guimarães and Calil, 2008; Linde-Arias et al., 2008; Veiga et al., 2006). This may then have long-lasting sub-lethal effects on their ability to perform ecologically essential activities that rely on swimming. The same dose of trichlorfon also reduces tolerance of hypoxia in *O. niloticus* (Thomaz et al., 2009).

##### 4.2. Individual variation in sensitivity to trichlorfon had numerous physiological determinants

There was overwhelming evidence that the proximate mechanism for the decline in  $U_{crit}$  performance was an impairment to  $E_{swim}$ . This presumably reflected effects of the AChE inhibitor on skeletal muscle function, as demonstrated for slow-twitch oxidative muscle in coho salmon (Tierney et al., 2007). It was not possible to measure AChE

inhibition in muscle of the tilapia in this study, because of the need to perform multiple swim tests. Similar doses of trichlorfon are known, however, to cause significant inhibition of axial muscle AChE in *O. niloticus* (Guimarães et al., 2007). Presumably, inhibition of AChE will have impaired neuromuscular coordination (Tierney et al., 2007) such that more ATP (and oxygen) was required to power swimming at any given speed, and the tilapia reached their AMR and then fatigued at a lower final speed. Although the extent to which trichlorfon reduced an individual's  $U_{crit}$  depended upon the extent to which it impaired their  $E_{swim}$ , it is not clear why some individuals showed almost no decline in  $U_{crit}$  and others showed a collapse in swimming performance of over 40%. The evidence that an individual's intrinsic  $E_{swim}$  could determine how well they performed after pesticide exposure, although not quite statistically significant, was nonetheless coherent with impaired  $E_{swim}$  being the main mechanism for compromised performance. This remains an interesting question for future study.

Given the reports that OPs can reduce metabolic rate, cardiac activity and spontaneous swimming activity in fishes (da Silva et al., 1993; Gehrke, 1988), it was perhaps unexpected that trichlorfon had no effects on SMR or AMR in the tilapia. One possible explanation is that these previous effects (Gehrke, 1988) reflected behavioural responses to the OP (Tryfonos et al., 2009), such that a decline in spontaneous activity lowered oxygen demand (da Silva et al., 1993). As stated already, however, this dose of trichlorfon compromised an effective hyperventilatory response to hypoxia in *O. niloticus* (Thomaz et al., 2009). In the current study, the tilapia probably used ram ventilation when swimming at high speeds (McKenzie et al., 2003) such that any reduced capacity to actively hyperventilate did not influence their ability to achieve their AMR. It was interesting that, although AMR was not affected by exposure to trichlorfon, individual  $U_{crit}$  swim performance was, nonetheless, strongly linked to AMR across all the trials. The strong negative relationship between AMR and % decline in  $U_{crit}$  performance after trichlorfon exposure may indicate that individuals which were able to achieve a high AMR were able to offset negative effects of the OP on their  $E_{swim}$ . It has been shown that AMR can be linked to intrinsic factors, such as cardiac physiology (Claireaux et al., 2005), and also to individual plastic responses to environmental conditions, such as nutritional state or training (Norin and Clark, 2016). It has been suggested to have links to individual fitness (Metcalfe et al., 2016). Given the significance of AMR for  $U_{crit}$  performance, it was surprising that intrinsic AMR, measured prior to OP exposure, was not a predictor of  $U_{crit}$  after OP exposure. These results require further research, not least to better understand causal relationships between AMR and  $U_{crit}$  in fishes.

The fact, that intrinsically better swimmers remained better swimmers after trichlorfon exposure, demonstrates that individual exercise performance is consistent across contexts in the Nile tilapia. This is interesting because the reasons for individual variation in swimming performance within fish species are not understood. It has been suggested that performance may vary with other elements of physiology, such as growth rate, sex, endocrine state, gut function, parasite load, among others (Killen et al., 2014; Marras et al., 2013; Oufiero and Garland, 2009). The results clearly indicate that individual sensitivity to toxic effects of the pesticide was independent of intrinsic  $U_{crit}$  performance, as this latter had no bearing on the relative extent to which performance declined after trichlorfon exposure. Overall, the current results indicate that this OP may not alter the relative selective effects of factors related to aerobic swimming ability (e.g. migratory potential, foraging) unless the performance of all individuals is decreased to such an extent that they all become more likely to experience reduced fitness (Killen et al., 2013).

## 5. Conclusions

The results demonstrate that a major proximate mechanism for impaired exercise performance in tilapia exposed to an OP pesticide

was reduced  $E_{swim}$ , and not effects on respiratory metabolism. Individuals varied widely in their sensitivity to trichlorfon but the extent to which their  $U_{crit}$  performance declined depended strongly upon the extent of impairment to their  $E_{swim}$ . This may have reflected different degrees of inhibition of the AChE activity that supports muscle contraction. Further research is needed to understand why individual  $U_{crit}$  was strongly dependent on AMR but AMR was not affected by OP exposure. This may reflect, in part, the complex potential causal relationships between AMR and  $U_{crit}$  in fishes. Although intrinsically good swimmers clearly remained better swimmers after trichlorfon exposure, individual percentage decline in performance was independent of intrinsic  $U_{crit}$ , which underscores the need for further investigation into mechanisms underlying differential sensitivity to the OP pesticide.

Individual variation in sensitivity to pollutants deserves more research attention in fishes (Kolok et al., 1998, 2002; Sloman, 2007), to gain insight into potential effects of these stressors on the makeup of populations (Calow and Forbes 1998; Brown et al., 2009). It is important to investigate why individual aerobic swimming performance varies so greatly within fish populations (Marras et al., 2013; Oufiero and Garland, 2009) better to understand which types of individuals are likely to be selected against if pollutants impair their performance.

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