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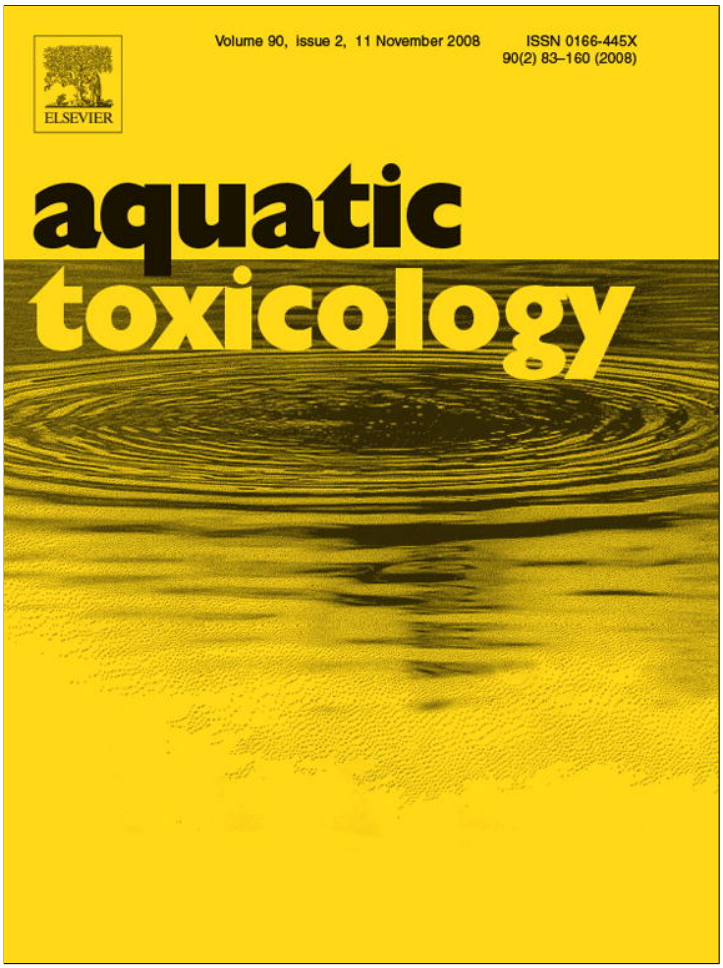

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# Social interactions, predation behaviour and fast start performance are affected by ammonia exposure in brown trout (*Salmo trutta* L.)

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ABSTRACT

In fish, fast starts are brief, sudden accelerations during predator–prey encounters. They serve for escape and predation and are therefore ecologically important movements. Fast starts are generated by glycolytic muscle performance and are influenced by many internal and external factors. It is known that ammonia pollution has a major effect on the glycolytic muscle action, thus creating conditions in which fast start performance might be reduced and predation rates altered. Therefore, escape response and predation strikes were investigated in brown trout (*Salmo trutta*) of 10 and 20 cm body length exposed to an elevated (1 mg $l^{-1}$) ammonia concentration for 24 and 96 h. Various locomotor and behavioural variables were measured. In C-starts, i.e. an escape start where the fish bends into a C-shaped position, ammonia exposure had no effect on response latency. After 96 h of exposure, cumulative distance, maximum swimming speed and turning radius of the prey were all significantly reduced and the escape went in no definite direction. The effect of ammonia exposure was more pronounced in large fish than in small fish. Predation strikes were also affected. Distance, speed and turning radius were significantly lower in exposed fish. Agonistic behaviour of dominant fish was significantly reduced and fish spent more time resting. Predator behaviour was also altered and the number of prey captured was reduced.

This study shows that ammonia exposure affects brown trout escape response mainly through a reduction in fast start velocity and through an impairment of directionality. Thus, in addition to a reduced strength of the response, ammonia exposure could also reduce the fish's elusiveness facing a predator. Predation rate and social interactions are disrupted and predator–prey relationships could be altered.



## 1. Introduction

Fast starts are highly energetic swimming bursts, started either from rest or imposed upon periods of steady swimming (Jayne and Lauder, 1993; Domenici and Batty, 1994). They are ecologically important movements in fish since they are used for escaping predators and for prey capture. Kinematically, different types of fast starts can be distinguished: (1) C-starts, which are generally used for escape responses, where a fish takes a C-like body shape before bursting, resulting from a contraction of the lateral musculature on the opposite side relative to the stimulus, and (2) S-starts which are used for escape but also predation, where an S-like shape can be observed, resulting from simultaneous contraction from the musculature on both sides (Domenici and Blake, 1997).

Predator–prey relationships are shown to be influenced by kinematics. Dill (1974) demonstrated that the prey's reactive distance increases with the speed and depth of the predator's body profile. A rapidly approaching predator may trigger an early response in the prey. Therefore, fast start speed of attacking predators is often sub-maximal (Webb, 1984; Harper and Blake, 1991). Also, predators aim at the centre of mass of their prey, perhaps because the centre of mass is the best target, since it can be located from the prey's geometry and it is also the point of a prey that moves the least during escapes (Webb and Skadsen, 1980). Domenici and Blake (1991) and Eaton and Emberley (1991) showed that turning angles in escape responses are variable but are mostly directed away from the attacking predator (Domenici and Blake, 1997). Striking angles of the predator may be species-specific (Hoogland et al., 1956) and are possibly influenced by gape limitation in addition to the prey's shape and other morphological factors, such as false eye spots (Domenici and Blake, 1997).

Fast starts are generated by white glycolytic muscles and are a movement of high intensity but short duration of muscle performance because of the ability of white muscles to generate high amounts of energy in a short time by means of anaerobic metabolism (Schulte et al., 1992). White muscles are known to be very susceptible to ammonia toxicity and it has been shown that ammonia exposure decreases swimming performance in fish

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(Beaumont et al., 1995; Shingles et al., 2001). Ammonia gas can diffuse into fish across the gills and therefore water $NH_3$ concentration determines the potential for toxicity (Shingles et al., 2001). The toxic effect of elevated ammonia concentrations in the water is attributed to a reduced outward flux of ammonia excretion through the gills, so that the outward flux is blocked and a reversed inward ammonia flux occurs. As a result, ammonia levels in the fish plasma increase. Another possible mechanism for plasma ammonia build-up at high external ammonia levels is the decreased ammonium outward flux through the $Na/NH_4^+$ exchanger (Wood, 1992). Beaumont et al. (2000a) suggested that increased $NH_4^+$ levels alter the metabolic status within the fish, arising from the effects on a number of metabolic pathways which may lead to premature muscle fatigue and, hence, a reduction in swimming performance. Also, measuring the resting membrane potential of white muscle Beaumont et al. (2000b) found a significant partial depolarisation. This was consistent with a predicted depolarisation, based on the measured distribution of ammonia between intracellular and extracellular compartments, and was suggested to be due to the displacement of $K^+$ by $NH_4^+$. As ammonium ions are allosteric activators of phosphofructokinase (Su and Storey, 1994) and inhibit pyruvate carboxylase (Zaleski and Bryla, 1977), elevated ammonia levels may increase the rate of flux through the glycolytic pathway, depleting stored glycogen levels and possibly also disrupting its regeneration in white muscles (Beaumont et al., 1995). High ammonia concentrations might, therefore, reduce fast start performance by impairing anaerobic capacity. Salmonids are known to be susceptible to even low ammonia concentrations in freshwater (Shingles et al., 2001).

The objectives of this study were to evaluate the effects of ammonia exposure on physiological and behavioural aspects of escape and predation in brown trout (*Salmo trutta*). The exposure concentration chosen (1 mg $l^{-1}$) represents the legal average quality norm (absolute quality norm: 5 mg $l^{-1}$) for surface waters in Flanders (Decision Flemish Government. 1 June 1995: (B.S. 31 July 1995)). Exposure times were altered to mimic the effect of short and long-term ammonia exposure on trout. An integrative approach was used in order to test the hypothesis that ammonia exposure has an effect on timing and locomotor variables of escape performance and predation fast start by integrating measure of fast start kinematics to investigate the implications of ammonia exposure on predator–prey interactions. Also, to investigate if such an effect could affect predator–prey interactions, predator behaviour and prey capture rate were considered.

## 2. Materials and methods

### 2.1. Fish

Brown trout of a body length ($L$) of 10 cm (10.01 ± 0.09 cm, hereafter referred to as 'small') and 20 cm (20.16 ± 0.27 cm, hereafter referred to as 'large') were obtained from a fish farm and transported to the University of Antwerp were they were held in 200 l tanks in groups of 15 large or ca. 100 small individuals in softened Antwerp City tap water at a constant temperature of 15.0 ± 0.4 °C for at least 4 weeks before experiments started (water properties—Hardness: 270 mg $CaCO_3$, Ca: 79.3 mg $l^{-1}$, Mg: 7.4 mg $l^{-1}$, Na: 27.8 mg $l^{-1}$, pH: 7.8 ± 0.1, $O_2$ > 90% sat). Tanks were in flow-through and water was partially renewed with a turnover rate of 100 l per day. Additional filtering occurred by means of a triple filter consisting of cotton, active carbon and lava stone. Fish were fed with Nutra Fish Food (Skretting, France) once a day. 72 h before the experiment started, fish were transferred to a 200-l flow-through tank in groups of two individuals and kept starved.

### 2.2. Exposure

The 96-h ammonia exposure started in the 200-l flow-through tank where groups of two fish were kept for 72 h before the experiment. The volume of 200 l was spiked with the required amount of an $NH_4Cl$ stock solution (Merck, Darmstadt, Germany), and subsequently ammonia solution was added by means of a peristaltic pump (Watson–Marlow, Falmouth, UK) in order to compensate for the water renewal. This resulted in a constant concentration of 1.09 ± 0.12 mg $l^{-1}$. For the 24 h exposure experiment, and for the last 24 h of the 96 h exposure experiment, exposure took place in the experimental tank (see below). Groups of two fish were exposed together and used together in the experiments.

A second set of fish was exposed in the flow-through tanks as described above, and after 24 and 96 h of exposure fish were netted and quickly killed in an overdose of buffered MS-222 (1 g $l^{-1}$, pH 7.4, Acros Organics, Geel, Belgium) in order to determine accumulated ammonia in blood and white muscles. Fish were immediately put on ice and a blood sample was drawn from the caudal blood vessel. Blood was immediately centrifuged for 3 min in 1.5 ml bullet tubes (13,200 × $g$) and snap-frozen in liquid nitrogen. In the mean time, a small piece of white muscle was dissected and snap-frozen in liquid nitrogen as well. Samples were later stored at −80 °C for ammonia analysis.

### 2.3. Experimental setup

Experiments were always conducted with two fish because it increased activity and therefore the probability that one of the fish was at the centre of the tank (under the camera). 24 h before the experiment two large fish were transferred to a square tank (120 cm × 120 cm) and two small fish were transferred to a round tank (95 cm diameter). Water depth was 20 cm and temperature was 15.0 ± 0.4 °C. A black curtain prevented disturbance of the fish. In exposure experiments, the water had an ammonia concentration of 1.02 ± 0.10 mg $l^{-1}$. Control groups were not exposed to ammonia.

A high-speed camera (Redlake Imaging Motion Scope PCI 1000 S) was used to record the fast starts at 500 fps (frames per second). The camera was positioned 2.1 m above the experimental tank. Video recording started ca. 1 s before stimulus and lasted for 3 s.

Escape responses were elicited by triggering the fall of a dummy (black PVC cylinder with a tapered tip, 2.8 cm diameter, 11.0 cm length) held 1 m above the experimental tank by an automatic release construction. In order to provide a sudden stimulus and allow calculation of the response timing, the dummy passed through a black tube (110 cm × 4 cm) with its lower edge 5 mm above the water surface. In order to determine impact timing, the dummy was dropped into the water above a mirror and the impact was recorded by high-speed filming (500 fps, Lefrancois et al., 2005) with the propagating wave being visible outside the tube 2 ms after impact and so it was taken into account that the stimulus ($t_0$) occurred 2 ms before the wave was visible. In order to avoid pseudoreplication only 1 fish of the 2 was chosen for further analysis. Not all fish immediately responded with a clearly defined escape response (see Section 3). Therefore, sufficient sets of fish (10–14 sets of 2 fish) were tested so that 10 clear double bend escape responses could be analysed, resulting in $N$ = 10 for all statistical analysis. The distance between the large fish varied from approximately 15 to 120 cm and between the small fish from approximately 10 to 70 cm and was not significantly different between control and exposed groups at the start of the recording. However, the individual fish being analysed was chosen as being the nearest to the stimulus and also the first one to respond in all cases, at a distance to the stimulus of approximately 5–20 cm.

**Table 1**
Measurements and definitions of kinematic factors in escape and predation fast starts

| Measurement | Definition |
|---|---|
| Responsiveness | Percentage of the animals tested to performing escape response upon presentation of stimulus |
| Response latency | Time interval between stimulus onset ($t_0$ as defined above) and first detectable movement of the escape response ($t_1$) |
| Duration: (a) stage 1 duration; (b) stage 2 duration; (c) total duration | (a) Time interval between the moment of $t_1$ and the change in direction of the head ($t_2$); (b) beginning at $t_2$ and ended when a further reversal of head turning direction occurred (Domenici and Blake, 1997); (c) (a) and (b) |
| Directionality: (a) away; (b) towards | Fast starts were divided into 'away' and 'towards' responses on the basis of $t_1$ being oriented away or towards the stimulus, respectively |
| $A_0$ | Initial orientation angle: angle between the line passing through the stimulus position and CoM ($D_0$) and the line passing through CoM and the tip of the head of the fish at the onset of the escape response $t_0$ ($D_{S0}$, Fig. 1). $A_0$ was transformed, so that stimulus position was always considered to be on the right side of the fish, thus $A_0$ ranged from 0 to 180 (Domenic and Blake, 1993; Domenici, 2002) |
| $A_1$ | Stage 1 angle: angle between $D_{S0}$ and the line passing through the CoM and the head of the fish at the end of the stage 1 ($D_{S1}$, Fig. 1) |
| $A_2$ | Escape trajectory: angle between $D_0$ and the line passing through the CoM and the head of the fish at the end of the stage 2 ($D_{S2}$, Fig. 1). $A_2$ was transformed, so that stimulus position was always considered to be on the right side of the fish, thus $A_2$ ranged from 0 to 360° (Domenic and Blake, 1993; Domenici, 2002). |
| Response types: (a) single bend (SB); (b) double bend (DB) | In DB responses, both stage 1 and stage 2 occur. For SB responses, stage 2 does not take place, since at the end of stage 1, fish straighten and glide |
| Average turning rate | $A_1$ divided by the time taken to complete stage 1. The instantaneous turning rate was determined as the difference between the angles of two consecutive frames divided by the corresponding time interval (i.e. 2 ms) |
| Minimum turning radius | Smallest radius of the approximately circular path of the CoM during an escape fast start |
| Distance time variables: (a) $D_{tot}$; (b) $U_{max}$; (c) $A_{max}$; (d) $D_{100}$ | (a) Total distance, (b) maximum speed and (c) maximum acceleration of the total fast start (stage 1 plus stage 2 durations). (d) Distance covered by each fish within 100 ms after the stimulus onset, considering both response latency and cumulative distance, as calculation of distance-related variables within a given time was suggested by previous authors (Webb, 1976; Domenic and Blake, 1993) to avoid any performance bias due to differences in fast start duration (Lefrancois et al., 2005). The time frame of 100 ms always fell well within the total duration of the fast start |

Predation fast starts were triggered by means of live bait. A thin string (2.2 m long) was tied to a small carp's ($L$ 3.4 ± 0.2 cm) abdomen behind the pectoral fins without any surgical procedure and the string was fixed at a height of 2.1 m so that the carp was swimming in small (ca. 30–40 cm diameter) circles under the camera. Two large trout were introduced and feeding events were recorded with a high-speed camera (see above). Fish were tested in sets of two, of which only the fish that actually ate the prey was chosen for further analysis (i.e. only 10 for analysis).

In another experiment, behavioural observations were conducted using a PAL video camera (Sony Corporation DCR-HC39E) positioned above the tank at a height of 2.1 m. Five small carp were introduced to the tank where they would swim freely and the behaviour of 2 large trout was recorded for 6 consecutive hours. In total, 20 trout and 50 carps were used, i.e. 10 replicates with 2 trout and 5 carps per replica.

### 2.4. Analysis

Water ammonia levels were determined using the salicylate-hypochlorite method according to Verdouw et al. (1978). Total muscle ammonia was extracted according to the method described by Wright et al. (1995). Total muscle and plasma ammonia was measured using an enzymatic kit (r-Biopharm, Boehringer Mannheim Darmstadt, Germany).

The centre of mass (CoM) of the trout was determined by hanging dead frozen fish from two different points (in front of the dorsal fin and at the cloaca) and determining the crossing point of the vertical extension of the two lines. Eight brown trout of each size class were used and the position of the CoM was determined to be at 0.45 ± 0.01 L from the tip of the head for the large fish and at 0.40 ± 0.04 L for small fish (total length of the fish = 1).

Measurements of orientation and locomotor variables were made following the methodology described by Domenici and Blake (1991) and Domenic and Blake (1993). The XY coordinates of the centre of mass and the tip of the head were digitised for each fast start sequence from video sequences (AVI files) exported into Vernier logger pro 3.4.6 (Vernier Software & Technology, TX) to perform a manual tracking of the fish's movements. Locomotor variables were calculated using the digitised coordinates, on which a five points smoothing polynomial regression procedure was applied for each derivative step (Lanczos, 1956) and the parameters assessed are listed in Table 1.

The analysis of the predation fast starts included the following variables $U_{max}$, $A_{max}$, $D_{tot}$ and $D_{100}$ (Table 1).

For behavioural observations the duration of behavioural traits listed in Table 2 were measured and the number of prey captured as well as success ratio, i.e. the number of feeding events per number of predation attempts was counted.

### 2.5. Statistics

All assumptions for parametric tests (normality of data) were tested and met.

**Table 2**
Behavioural traits and definitions

| Behavioural traits | Definition |
|---|---|
| Resting | Time spent immobile |
| Agonistic behaviour | Time spent with intraspecific aggressive behaviour |
| Predation | Time period from moving towards prey until ingestion of prey |
| Swimming | Time spent moving freely in tank |
| Prey handling | Time spent feeding/number of feeding events |

*Escape response*: Responsiveness and the proportion of DB and SB responses were analysed using one-way ANOVA with a Bonferroni post hoc test. The significance level for all tests was determined at $p < 0.05$. To determine if ammonia exposure had an effect on directionality of response (i.e. 'away:towards'), a two-tailed binominal test was performed. Since $A_2$ determined the final direction of the escape, circular statistics (Mardia–Watson–Wheeler test, *W*-test; Lefrancois et al., 2005) was used to assess the effect of ammonia exposure on $A_2$ and other directional variables, such as initial orientation and the angle between stimulus and initial orientation. The effect of size, ammonia and exposure time on the locomotor performance variables ($U_{max}$, $A_{max}$, latency, duration, turning rates, minimum turning radius, $D_{tot}$ and $D_{100}$) was analysed using multiple ANOVA with a Bonferroni post hoc test. The significance level for all tests was determined at $p < 0.05$.

*Predation fast starts*: The effect of ammonia and exposure time on the locomotor performance variables ($U_{max}$, $A_{max}$, duration, $D_{tot}$ and $D_{100}$) was analysed using two-way ANOVA with a Bonferroni post hoc test. The significance level for all tests was determined at $p < 0.05$.

*Observations of predation behaviour*: The effect of ammonia and exposure time on behavioural traits, numeral and temporal parameters, was analysed using two-way ANOVA with a Bonferroni post hoc test. The significance level for all tests was determined at $p < 0.05$.

All experiments complied with the regulations of the Ethical Board of the University of Antwerp.

## 3. Results

### 3.1. Escape performance

Responsiveness in small (10 cm) fish was 100% ($N = 10$) in all cases, as it was in large fish in control and 24 h of exposure ($N = 11$). In 96 h of exposure, the responsiveness was reduced to 85.71% ($N = 12$ out of 14) in large fish. However, this decrease was not significant.

Of the fish that responded, the proportion of DB type responses in small (10 cm) fish was 100% in all cases ($N = 10$). In large (20 cm) fish the proportion of DB type responses was 90.91% ($N = 10$ out of 11) in control and 24 h exposed fish and 83.33% ($N = 10$ out of 12) in 96 h exposed fishes. The difference was not significant. Only the reaction of the fish next to the trigger was taken into consideration. However, because too few SB reactions were noted, statistical analysis of C-start factors was only carried out on the DB responses (Fig. 1).

Results of C-start kinematics analysis show that in small fish the maximum velocity ($U_{max}$) significantly decreased after 96 h of ammonia exposure as it did in large fish (Fig. 2a). Maximum acceleration ($A_{max}$) also shows a significant difference between the treatments (Fig. 2b). There was no difference between the size classes.

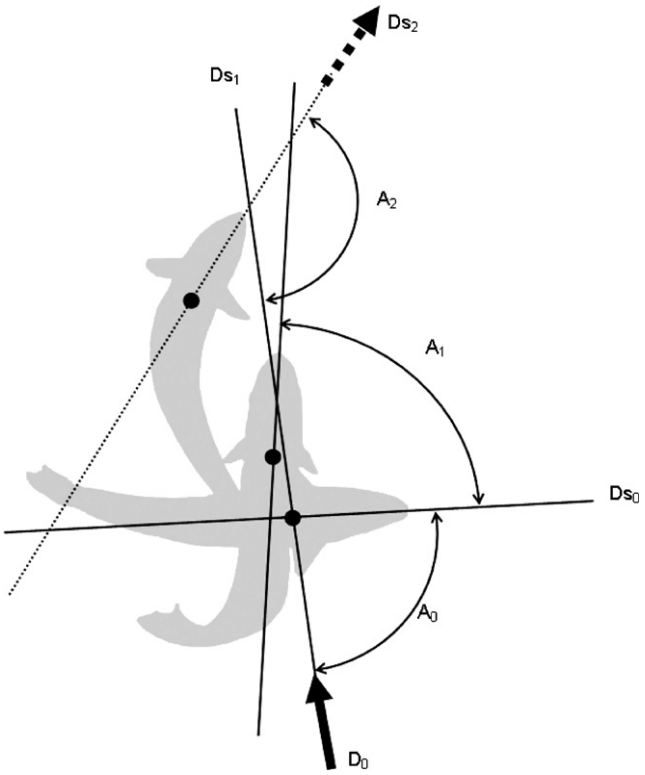


**Fig. 1.** Illustration of the angular variables (solid arrow: stimulus direction; dotted arrow: escape direction). $D_0$, line passing through the stimulus position and the fish centre of mass (CoM); $D_{S0}$, line passing through the CoM and the head of the fish at the onset of the escape response; $D_{S1}$, line passing through the CoM and the head of the fish at the end of the stage 1; $D_{S2}$, line passing through the CoM and the head of the fish at the end of the stage 2; $A_0$, initial orientation; $A_1$, stage 1 angle; $A_2$, escape trajectory.

Total duration of C-starts increased in both size classes after 96 h of exposure and differed between the size classes. Here, only stage two contributed significantly to the differences (Fig. 2c). Total distance ($D_{tot}$) and distance over 100 ms ($D_{100}$) show similar effect results. $D_{tot}$ in C-starts differed significantly between 0 and 96 h of exposure as did $D_{100}$ in both, small and large fish (Fig. 2d). Also, there was a size effect on $D_{tot}$ and $D_{100}$ with higher values in large fish. No agonistic behaviour was observed between the small individuals.

Directionality analysis in small fish revealed an "away: towards" response ratio of stage 1 that was mainly away in small and in large fish in all conditions (pooled data: 90% away and 80% away, respectively). There was no variation of the angle between stimulus and initial orientation between the conditions (angular mean ± angular deviation). After exposing small fish to ammonia for 96 h, fish showed no significantly defined escape trajectory, while controls and exposure for 24 h revealed trajectories of $166.82 \pm 43.99°$ and $169.24 \pm 51.89°$, respectively (angular mean ± angular deviation). Large fish showed defined escape trajectories only before exposure, with a value of $171.12 \pm 37.70°$.

Average turning rate in small fish after 96 h of exposure ($2.46 \pm 0.25° \text{ ms}^{-1}$) differed significantly from control ($3.21 \pm 0.10° \text{ ms}^{-1}$) as did minimum turning radius after 96 h of exposure ($1.99 \pm 0.05$ cm) from control ($1.84 \pm 0.05$ cm). In large fish, average turning rate was significantly lower after 96 h of exposure ($1.99 \pm 0.14° \text{ ms}^{-1}$) compared to control ($3.21 \pm 0.10° \text{ ms}^{-1}$, Fig. 2e). The minimum turning radius was significantly increased after 96 h of exposure in both small and large fish compared to the respective controls. There was no significant difference in

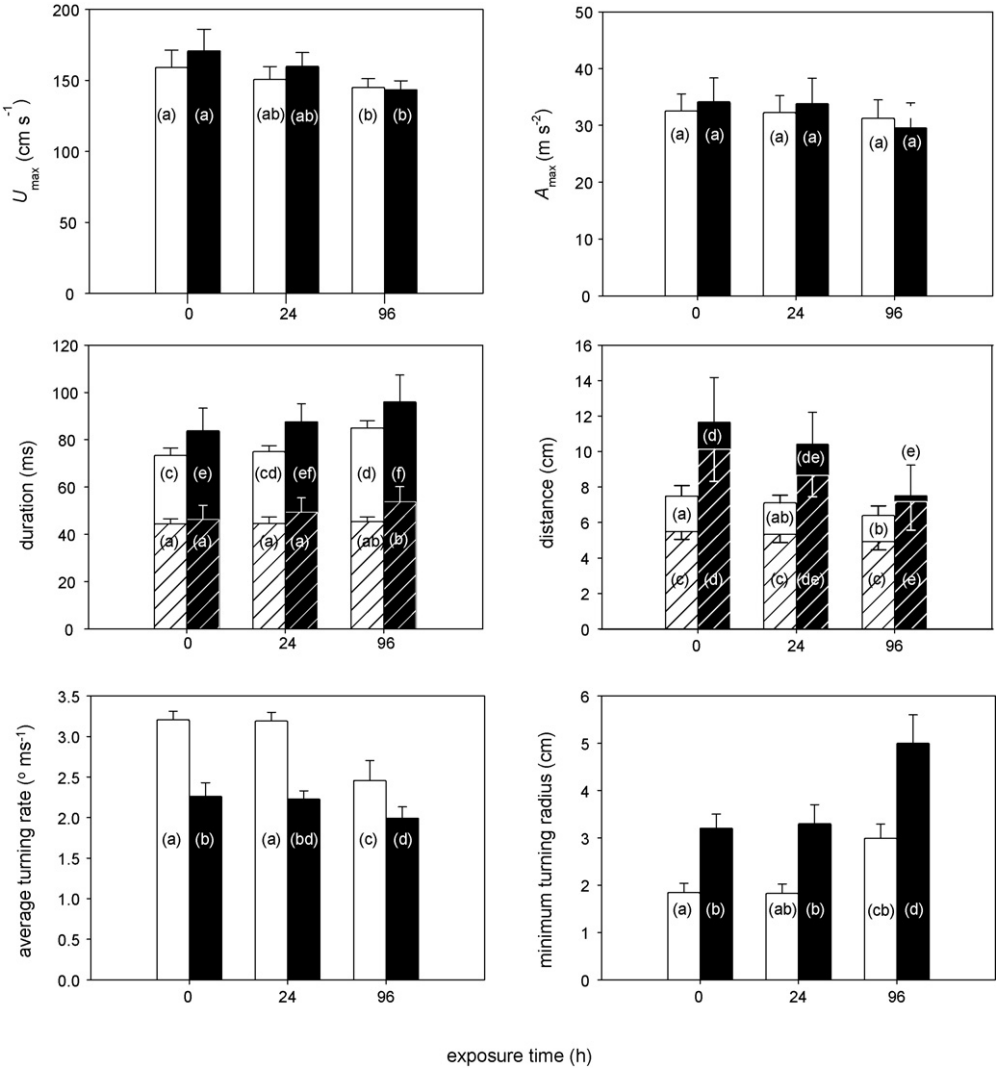


**Fig. 2.** Kinematic data of escape performance in brown trout. Maximum velocity ($V_{max}$, black) and acceleration ($A_{max}$, white) in small (a) and large (b) brown trout; duration of stage 1 (black) and stage 2 (white) of a C-start in small (c) and large (d) brown trout and total distance (white); distance over 100 ms (black) in small (e)) and large (f) brown trout after no exposure, 24 and 96 h of exposure. Values are mean ± S.D. *Indicates significant difference from control. Difference was significant at $p < 0.05$.

latency between exposure time or size, with a pooled value of 39.43 ± 7.86 ms. There was also no significant variation in the angle between stimulus and initial orientation.

### 3.2. Predation fast start

Both trout remained stationary, mostly at a side of the tank when the carp was introduced to the setup. The carp showed distress upon introduction but calmed down after a few minutes. Its mobility was limited by means of a string; therefore it could not turn in larger circles than approximately 30–40 cm. Approximately 30 min after introduction, one trout approached with an average velocity of 0.94 ± 1.2 Ls$^{-1}$. Carp attempted to escape when the approaching trout reached a distance of 21.24 ± 9.33 cm. A few seconds before burst swimming was noted, trout approached the carp by coasting. Bursts consisted of several beats with the tail fin, resulting in massive acceleration, and indicating the beginning of the predation fast start. The type of the fast start was similar in all cases with maximum velocity and acceleration reached at or slightly before the trout reached the position of the prey. The number of intense tail beats differed between 2 and 4, depending on the total distance of the fast start. Maximum velocity and acceleration were reached at or slightly before the trout reached the position of the prey. Kinematic variables of trout predation starts showed significant differences between treatments. Maximum velocity was significantly reduced after 96 h of exposure, and so was maximum acceleration (Fig. 3a). Total duration was significantly increased after 96 h of exposure (Fig. 3b). Also the distance over 100 ms was reduced after 96 h of exposure (Fig. 3c). There were no differences in the kinematic variables of the carp escape response between ammonia treatments (general values, mean ± S.D.—$U_{max}$: 0.51 ± 0.08 ms$^{-1}$, $A_{max}$: 19.21 ± 2.90 ms$^{-2}$).

### 3.3. Behavioural observations of predator–prey interactions

The results of the observations are given in Table 3. In general, the behaviour of the dominant individual was altered after 96 h of exposure. The time spent resting was increased ($p < 0.001$) while the time devoted to agonistic traits by the dominant towards the submissive individual were decreased ($p < 0.001$). Total predation time was decreased ($p < 0.01$). The time spent on cruising through the tank was decreased ($p < 0.05$) and similar to the result of the subordinate individual in control and after 24 h of exposure. Prey handling time was increased ($p < 0.05$). The prey capture rate was

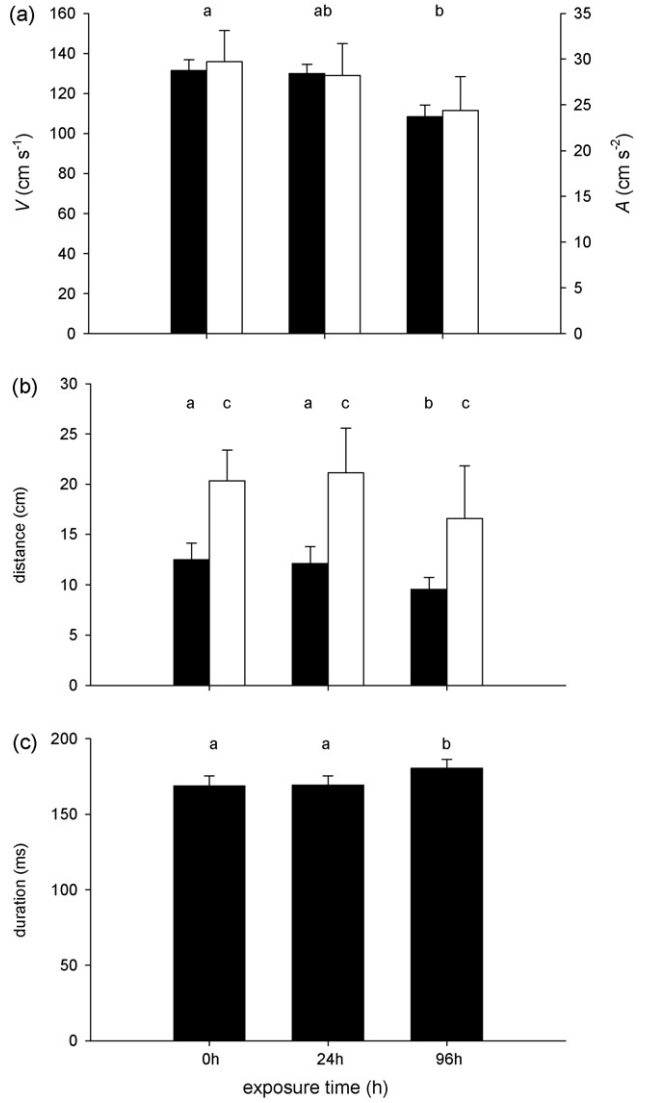


**Fig. 3.** Kinematic data of predation strike in large (20 cm body length) brown trout with (a) maximum velocity ($V_{max}$, black) and acceleration ($A_{max}$, white), (b) distance over 100 ms (black) and total distance (white) and (c) duration after no exposure, 24 and 96 h of exposure. Values not sharing a common script are significantly different. Difference was significant at $p < 0.05$.

decreased ($p < 0.05$) but success rate did not differ significantly. The average swimming velocity of the dominant trout was $15.70 \pm 4.09$ in control, $14.90 \pm 5.73$ after 24 h and $14.69 \pm 4.46$ after 96 h of exposure. The difference is not significant.

The subordinate individual showed no significant differences between exposure levels in any trait escept of predation, prey handeling and number of prey captured due to the fact that predation was absent after 96 h of exposure.

### 3.4. Plasma and tissue ammonia

Plasma and muscle ammonia were significantly elevated in small trout exposed for 96 h and in large trout exposed for 24 and 96 h to ammonia (Table 4).

## 4. Discussion

The present study shows the effects of increased ammonia concentration on escape and predation performance in brown trout. It clearly shows that an increased ammonia concentration in freshwater significantly reduces escape performance in small and large brown trout and therefore the chance to escape from the attack of an approaching predator is decreased. Also, predation fast start performance is reduced in large fish by high ammonia levels impairing kinematic factors such as velocity and acceleration and thus lessens the success of an attack. Finally, ammonia has an effect on predator–prey interactions by disrupting predation behaviour, changing intraspecific interactions in the predator and leading to the reduction of the predation rate. These findings show that an increased ammonia concentration in freshwater can significantly change predator–prey interactions in piscivorous communities.

To our knowledge, this is the first study that highlights the influence of ammonia or another toxin on predator–prey interactions from an interactive angle. The importance of this study relies in the fact that it shows not only the effects on the prey but also the effects on the predator and finally functional and numeral effects on predator–prey interactions. Additionally, it is shown that even a low ($1\ mg\ l^{-1}$) ammonia concentration in freshwater can lead to a significant impairment of predator–prey interactions.

### 4.1. Escape performance

Directionality of escapes and/or predation fast starts can be discussed with respect to three parameters: initial orientation of the escape response, i.e. 'towards' or 'away', average turning rate and final orientation. The results on directionality in small fish escape responses showed to be mainly 'away' from the direction of the stimulus (i.e. between 90 and 270°) in clean water and after 24 h of exposure, but large fish show 50% 'towards' responses already after 24 h of exposure. The final orientation of the escape response of unexposed trout of both size classes and small trout after 24 h of exposure showed a significant average around 170° from the stimulus. Large trout respond randomly after an exposure of 24 h, while small trout respond randomly only after 96 h of exposure. This is an indication of a negative effect of ammonia exposure on the success of escapes in large trout already after a relative short period of time. According to the assumption that small fish are generally more influenced by environment than large fish because of their

**Table 3**
Behavioural traits of a dominant and a subordinate individual predating on 5 small carps for 6 h, success ratio and number of prey captured (mean ± S.D., significantly different from control: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$)

| Exposure time (h) | Dominant individual | | | Subordinate individual | | |
|---|---|---|---|---|---|---|
| | 0 | 24 | 96 | 0 | 24 | 96 |
| Resting (min) | 222.99 ± 22.74 | 228.24 ± 44.39 | 254.88 ± 32.84*** | 280.18 ± 21.11 | 277.92 ± 13.87 | 262.88 ± 17.86 |
| Agonistic behaviour (min) | 73.26 ± 7.97 | 68.12 ± 7.24 | 52.73 ± 13.46*** | 0 | 0 | 0 |
| Predation (s) | 31.10 ± 7.37 | 31.50 ± 6.63 | 18.90 ± 2.23** | 8.00 ± 1.00 | 6.75 ± 0.51 | 0 |
| Swimming (min) | 83.70 ± 28.14 | 79.73 ± 42.11 | 31.54 ± 32.29* | 27.03 ± 19.58 | 30.57 ± 16.78 | 23.92 ± 14.39 |
| Prey handling (s) | 7.44 ± 2.25 | 8.12 ± 2.55 | 10.91 ± 3.98* | 7.00 ± 1.00 | 7.75 ± 1.89 | 0 |
| Success ratio | 0.96 ± 0.08 | 0.89 ± 0.11 | 0.86 ± 0.21 | 0.75 ± 0.50 | 0.80 ± 0.44 | – |
| Number prey captured | 4.30 ± 0.48 | 4.00 ± 0.47 | 1.90 ± 0.56*** | 0.30 ± 0.48 | 0.40 ± 0.51 | 0 |

**Table 4**
Plasma and white muscle ammonia level in brown trout after exposure to 1 mg $l^{-1}$ ammonia for 24 and 96 h or control (0 h)

| Exposure time (h) | Ammonia content white muscle (μmol $g^{-1}$) | | | Ammonia content plasma (μmol $l^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 0 | 24 | 96 | 0 | 24 | 96 |
| Small individuals | 1.22 ± 0.21 | 1.52 ± 0.24 | 1.74 ± 0,45[a] | 124.6 ± 32.57 | 386.49 ± 56.74 | 401.43 ± 62.58[a] |
| Large individuals | 1.53 ± 0.33 | 1.97 ± 0.35[a] | 2.03 ± 0.53[a] | 153.54 ± 34 | 412.76 ± 45.42[a] | 487.76 ± 54.24[a] |

[a] Significantly different from control (mean ± S.D., $p < 0.05$).

increased surface volume ratio, the results would be expected to show a stronger effect on small fish.

Small and large brown trout in the control group reached maximum velocity ($U_{max}$), maximum acceleration ($A_{max}$) and a total duration ($D_{tot}$; see Table 1) values similar to those found by Webb (1976) in rainbow trout (*Oncorhynchuss mykiss*). The present study shows that ammonia exposure for 96 h in large and small fish significantly reduces $U_{max}$ in fast starts. From an ecological point of view, it can be stated that a reduced fast start performance can have an effect on escape and predation. Walker et al. (2005) showed that faster fast starts increase the probability of evading predators. Thus, a reduced speed could lead to a higher risk to be captured by a predator. Consequently, predators, not affected by ammonia concentrations in the water, like avian predators or mammals, could have a significantly increased success in predation on brown trout in ammonia polluted freshwater. Other variables tested showed no significance differences under the influence of ammonia. The latency of an escape was similar in both exposed and unexposed fish. This implies that ammonia does not have an impairing effect on the perception of the stimulus and the timing of Mauthner cell action. Also, there was no effect of ammonia on responsiveness, the number of individuals responding to the stimulus and therefore relevant for obvious reasons. This factor was strongly influenced by hypoxia (Lefrancois et al., 2005).

Comparison of the data according to body size shows that maximum C-start velocity is related to body size. This is in accordance with Wardle (1975), who showed that burst speed is related to minimum muscle contraction time, being in turn related to fish size. Also, total distance of a fast start is size-dependent because bigger fish perform longer fast starts (Webb, 1976). However, it is shown that the effect of ammonia on the startle response of small and large trout is disrupting to a degree that fish can become an easier prey by reducing kinematic values and impairing directionality. Also, this effect is stronger on large than on small fish.

### 4.2. Predation performance

Results on predation fast start variables show a decrease in $U_{max}$ and $A_{max}$ after 96 h of exposure. Also, the distance over 100 ms ($D_{100}$; Table 1) is significantly reduced but not $D_{tot}$. This is due to the increased duration of predation fast starts after 96 h of exposure but not the distance to the prey when the fast start was elicited. However, as $D_{100}$ is the variable that has more effect on possible predation success (Webb, 1976; Domenic and Blake, 1993), the results show a negative effect of ammonia exposure on predation performance. As performance of escape fast start is reduced with exposure to an increased ammonia concentration, this should affect predator–prey relationships in nature. Walker et al. (2005) showed that faster escape fast starts increase the probability of evading a predator. Thus, predators have to strike fast in order to be successful. The present study shows that exposure to ammonia reduces not only escape but also predation fast start performance. Therefore, it can be assumed that a reduction of predation fast start performance can lead to a lower success rate in nature, especially when fast start performance of the prey is not affected by ammonia concentration in the water. To our knowledge, there are not many studies on predation fast starts. The few studies published (e.g. Webb and Skadsen, 1980; Rand and Lauder, 1981; Harper and Blake, 1990, 1991) present similar values on feeding strikes as those found in the present study. Even though these studies present feeding S-starts in pikes (Genus *Esox*), our results on trout show feeding strikes that are very similar to a feeding strike observed by Harper and Blake (1991, feeding fast start IV). However, as the feeding strike did not have much similarity to an S-start in rainbow trout described by Webb (1976), seeing the high $U_{max}$ values reached it still can be generated by means of white muscle action without being innervated by Mauthner cells. According to Domenici and Blake (1997) feeding fast starts are unlikely to be generated by Mauthner cells because of their repetitive movement patterns.

The exposure to ammonia at high concentrations could also influence the hunger level and therefore reduce the motivation to attack prey. This would explain the delayed predation action after exposure when presenting the trout with the bait. However, the starvation time of 96 h should be sufficient to elicit a predation fast start with maximum performance, also indicated by maximum velocity and acceleration values found that are comparable to maximum values at escape fast starts in our study and in the literature (e.g. Webb, 1976).

### 4.3. Observations of predator–prey interaction

Studies about fast start and escape performance are generally one-sided, highlighting only the performance of the prey, and ignoring possible effects of experimental factors (e.g.: hypoxia, toxins, reduced visibility) on the predator (e.g. Lefrancois et al., 2005; Meager et al., 2006; Weber, 2006). Thus, it can be argued that there might be no change in encounter rate and mortality of the prey population as the performance of the predator is altered in the same way. However, the ammonia concentrations used in this study did not show any impairing effect on the swimming capacity observed in the prey, i.e. the common carp. Israeli-Weinstein and Kimmel (1998) showed that the concentration used (1 mg $l^{-1}$) is not influencing swimming behaviour in carp similar to the size used in the present study. Moreover, observation and monitoring of behaviour of the predator reveals that increased ammonia concentration alters intra- and interspecific behaviour and thus reduces predation rate and mortality of the prey. Therefore, as brown trout is known to predate while migrating (Lucas and Baras, 2001), an impairing effect of increased ammonia concentrations on predator–prey interactions can be analysed only specifically with respect to the predator–prey couple.

As shown, trout is a sensitive fish and swimming capacity is reduced (Shingles et al., 2001; Wicks et al., 2002; McKenzie et al., 2003) at a low ammonia concentration that does not affect other species like carp in our study. Thus, as different species react differently to the same concentration of ammonia in the water, it can be assumed that also predators of trout might be affected differently (e.g. lampreys and pike) or not at all (e.g. birds, seals and sea lions).

Observations reveal that agonistic and predation behaviour of trout is reduced with increased ammonia concentration in the water. Quigley (1975) showed that an increased ammonia concentration reduced the number of agonistic acts between

individuals of rainbow trout, confirmed in brown trout by the present study. Israeli-Weinstein and Kimmel (1998) reported that exposure to high ammonia concentrations altered behaviour in carp. The present study shows that inter- and intraspecific behaviour is significantly altered in brown trout with the result that hierarchical differences are reduced and that the numeral and functional effect of predation is altered. Ecologically, these finding have severe implications on group and population dynamics. The change in interactions between members of the same group can lead to loose and dysfunctional group structure and finally have an effect on mating choice and reproductive success, while the reduced predation rate influences the interactions of two species in an ecotope and leads to the disruption of population dynamics.

### 4.4. Ecological relevance

The concentration of 1 mg $l^{-1}$ used in this study is the concentration is the Belgian Water Quality Criterion for surface waters. According to the Flemish Environmental Agency (Vlaamse Milieumaatschappij, www.vmm.be), the values found in Flemish water bodies often exceed this limit. This, among other factors, might explain why the population numbers of trout in Flanders have decreased (Dumortier et al., 2005). Moreover, Walloon populations might also be affected, as they use Flemish waterways to migrate towards the open sea. There are many other potential reasons acting in concert for the decline of European brown trout and ammonia alone is unlikely to be the cause. However, this study clearly shows the negative ecological impact of increased ammonia values on freshwater trout populations and therefore may partly explain decreased population numbers in nature.

As the surface to volume ratio is higher in small than in large animals, it can be expected that small fish are more susceptible than large fish. The results show that small fish are less susceptible for ammonia than large fish. Possibly, juvenile individuals still benefit from ammonia detoxification mechanisms during their embryonic phase (Steele et al., 2001). Alternatively, a higher metabolism in smaller individuals leads to a higher $CO_2$ excretion that reduces the pH at the gills. The surrounding water becomes more acidic leading to a higher amount of $NH_4^+$ which makes it easier for $NH_3$ to leave the body via passive diffusion, and/or harder for environmental ammonia to enter the gills (Wilson et al., 1994).

### 4.5. Tethered life prey

For the evaluation of the effect of ammonia on predation performance and the predator–prey interactions, tethered live bait was used. This fact and the fact that the tether consisted of a string tied to the carp's abdomen (without surgical attachment or under anaesthesia) can lead to concerns about the welfare of the fish.

However, the procedure of attaching the thread to the carp's body was brief and accurate and the fish was then free to swim. After a few minutes the carp stopped showing signs of acute stress such as excessive opercular movements, and erratic swimming behaviour or failure to swim at all were never observed. From a methodological viewpoint, predator behaviour and fast start performance can depend on prey species and behaviour and be the reason for suboptimal performance. The present study tries to show the effect of elevated ammonia concentrations on piscivorous communities. The choice for a non-vertebrate prey or a fisherman's 'fly' pulled through the water could impair the predation fast start and influence our results. A study most similar to the present one by Harper and Blake (1991) used a goldfish tethered by a threat attached to its lower lip. An alternative was not suggested.

We want to point out that alternatives to a life tethered prey were not available at the moment the study was performed. However, studies where accuracy to the millisecond of kinematic measurements is not required should use inanimate lures and baits or dead fish in order to avoid unnecessary suffering in the prey.

## 5. Conclusion

It can be concluded that a high ammonia concentration in freshwater leads to reduced fast start performance in brown trout. This has an effect not only on escape but also on predation performance and might therefore alter predator–prey interactions. Moreover, ammonia exposure alters the behaviour of the predator and thus may impair predator–prey relationships on a population dynamical level. The concentrations used in this study represent concentrations found in nature and are thus ecologically relevant. These effects of ammonia on fast start performance should, therefore, be considered when establishing guidelines for threshold concentrations.

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