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ARTICLE

Consequences of Air Exposure on the Physiology and Behavior of Caught-and-Released Common Carp in the Laboratory and under Natural Conditions

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Abstract

Specialized anglers of Common Carp *Cyprinus carpio* in Europe and increasingly in North America usually release trophy-sized fish, often following retention in carp sacks, to facilitate photographic memories. This practice is associated with extended air exposure. We assessed the impact of air exposure for a period of 10 min after capture and after an additional 9 h of retention in carp sacks on the physiology of small carp at two water temperatures (12°C and 22°C) under laboratory conditions. In a complementary field experiment with large carp, we also assessed the effects of air exposure on their physiology and additionally assessed the effects on tissue damage, postrelease behavior (i.e., movement and time rested), and survival. In the laboratory, plasma lactate increased by 24% during air exposure following simulated capture, and blood pH dropped by 0.16 units relative to a capture-only situation. Other physiological variables were unaffected by the treatment. In the field, air exposure after capture did not affect any physiological variables or indicators of tissue damage. During retention in carp sacks, fish recovered from capture, but subsequent air exposure caused a plasma lactate rise of 358% in the laboratory and 89% in the field experiment, and blood pH dropped by 0.38 units in the laboratory relative to that for the retained fish. In the field experiment,

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postrelease movement was significantly reduced in air-exposed fish, but normalized within 12 h. No mortalities occurred within 2 months postrelease. From a fish welfare perspective, our results suggest that photography should be conducted directly following capture without further retention in carp sacks as this approach is less deleterious to the fish. However, there is no benefit in doing so for maintaining carp populations because no substantial mortalities are to be expected. Overall, Common Carp are highly resilient to even extended air exposure.

In Europe, Common Carp *Cyprinus carpio* is among the most important species for recreational fisheries (Arlinghaus and Mehner 2003). In recent years, specialized carp angling for trophy-sized specimens has gained particular popularity and is conducted as a total catch-and-release fishery (Arlinghaus 2007). Specialized carp angling is also becoming increasingly popular in North America, where carp angler groups promote carp as a target species for recreational fisheries, encourage the release of trophy fish, and organize catch-and-release angling tournaments including world-wide recognized events such as the World Carp Championship in 2005 (Farooqi 2006; Quade 2009). Among the fundamental catch-related motivations in specialized carp angling is a documented photograph of a captured trophy specimen (Arlinghaus and Mehner 2003). Photography, in conjunction with weighing and measuring, typically results in prolonged periods of air exposure (i.e., often over 10 min according to personal observation and experience) as specialized carp anglers aim at taking pictures of both sides of the fish's body for future identification purposes (Arlinghaus 2007).

Air exposure represents one of the most critical stressors in catch-and-release angling (Cooke and Suski 2005; Arlinghaus et al. 2007) and can exacerbate fish welfare concerns (Huntingford et al. 2006). It causes a collapse of the gill lamellae and adhesion of the gill filaments, which collectively reduces the gill surface area and results in an inhibition of gas exchange (Boutilier 1990; Ferguson and Tufts 1992). The inhibition of gas exchange promotes alterations to the physiological status of fish similar to those evoked by capture, such as a pronounced neuroendocrine (i.e., primary) stress response, which modulates subsequent changes in blood and muscle biochemistry (i.e., secondary stress response; Ferguson and Tufts 1992; Haukenes and Buck 2006; Arlinghaus et al. 2009). Physiological alterations can affect the whole-animal performance (i.e., tertiary stress response), and several studies have demonstrated behavioral alterations after release (Cooke and Philipp 2004; Thompson et al. 2008; Arlinghaus et al. 2009). Ultimately, air exposure can increase mortality rates (Ferguson and Tufts 1992; Gingerich et al. 2007), which is, however, not always the case (Thompson et al. 2008; White et al. 2008; Arlinghaus et al. 2009) and is typically influenced by the magnitude of physiological disturbances. The degree of physiological and behavioral alterations and the recovery speed are correlated with the duration of air exposure (Ferguson and Tufts 1992; Schreer et al. 2005; Gingerich et al. 2007) and further influenced by additional stressors to which fish are exposed to during catch-and-release angling (Killen et al. 2003; Suski et al. 2003). In specialized carp angling, retention in carp sacks, which are collapsible, dark, knotless mesh bags made of synthetic fiber cloth, represents a common stressor (Rapp et al. 2012). Following capture carp are transferred into carp sacks, which are placed in shallow water, and carp are then retained for several hours if conditions do not permit immediate photography (e.g., during night). Rapp et al. (2012) demonstrated that carp-sack retention results in physiological and behavioral alterations, but concurrently, selected physiological disturbances (e.g., anaerobiosis) caused by capture are reversed. This also applies to other retention gear (e.g., keep nets, live wells) and other species if adequate water quality is maintained (Pottinger 1998; Suski et al. 2004; Killen et al. 2006). Due to the potential synergy of stressors, air exposure after retention in carp sacks may, however, result in interactive effects as was demonstrated for Largemouth Bass *Micropterus salmoides* and Walleye *Sander vitreus*, in which air exposure after live-well retention caused a second bout of anaerobiosis, which was similar to the initial capture (Suski et al. 2004; Killen et al. 2006). Similarly, interactions among various angling stressors and environmental conditions can affect the stress response and survival of released fish (Cooke and Suski 2005; Arlinghaus et al. 2007). Particularly the interactive effects of air exposure and high water temperatures have been found to be critical for the fish's viability in some studies (Davis and Schreck 2005; Gingerich et al. 2007). Angling for carp is usually conducted during spring, summer, and fall over a wide range of water temperatures, but the effects of extended air exposure at various temperatures on Common Carp during catch-and-release angling are unknown.

Some authors have reported consequences caused by angling beyond the typical stress response. This includes the leakage of intracellular enzymes (i.e., aspartate transaminase [AST] and lactate dehydrogenase [LDH]) into the blood stream (Morrissey et al. 2005; Butcher et al. 2011; Rapp et al. 2012), which is indicative of cell tissue damage as their function is restricted to the intracellular space and they are only released in the blood stream upon cell damage or death (Henry 1996). Enzyme leakage was also observed for carp during long-term carp-sack retention for up to 9 h (Rapp et al. 2012), but consequences of air exposure on tissue damage in this species are unknown and have only been studied for Largemouth Bass in which no significant effect of air exposure on tissue damage was observed (Thompson et al. 2008).

To date, there have been no studies on the sublethal and lethal effects of extended air exposure on Common Carp despite the

ubiquity of air exposure durations of up to 10 min or more in specialized carp angling at various capture temperatures. Furthermore, information on prolonged air exposure periods during catch-and-release angling is generally scarce as most studies focused on periods between 30 s and 3 min (e.g., Ferguson and Tufts 1992; Suski et al. 2004; Killen et al. 2006). Thus, this study also complements existing air exposure literature in catch-and-release angling science by adding observations of how Common Carp react to extended exposure to air.

This study aimed at assessing whether and to what extent prolonged air exposure resulted in a primary, secondary, or tertiary stress response, or stress-induced tissue damage and mortality. Moreover, the effects of high and low water temperatures on the stress response of air-exposed carp were examined to assess potential interactive effects of air exposure and water temperature. These questions were addressed using complementary laboratory and field experiments to take advantage of the strength of both approaches (Cooke et al. 2013). In the laboratory, effects of air exposure on the physiological status of age-2 Common Carp acclimated to two different water temperatures that resemble typical temperatures during spring and fall (12°C) and summer (22°C)—the main angling seasons for carp—were assessed. In the field, air exposure effects on fish physiology, tissue damage, short-term behavior (movement and time rested), and long-term fate of large carp as key targets of specialized carp anglers were assessed.

METHODS

Laboratory experiment.—Two hundred age-2 Common Carp were obtained from the Peitz commercial pond aquaculture facility (Schlepzig, Germany; 52°01′45.25″N, 13°53′43.60″E) on 16 October 2007. Fish were randomly distributed to four holding aquaria (~2,000 L each, 50 fish per tank, 10 kg/m^3) in a temperature-controlled laboratory at Humboldt-Universität zu Berlin, Germany. Each holding aquarium was equipped with a filtration system and additionally supplied with 60 L of tempered freshwater per hour. The initial water temperature was 18°C and was decreased to 12°C in two holding aquaria, and in the other two holding aquaria it was increased to 22°C by a maximum temperature change of 0.5°C per day during the first 2 weeks. The light regime was 11 h day : 1 h dusk : 11 h night : 1 h dawn. The carp were acclimated to laboratory conditions over a total period of 6 weeks prior to the start of the experiments (Pottinger 1998) and were fed a commercial carp diet twice a day at 2% body weight (Kraftfutter Beeskow GmbH, Beeskow, Germany; KM 28/08).

To assess the effects of air exposure on carp acclimated to both water temperatures, individuals were randomly allocated to one of five treatment groups: (1) minimally stressed as a control group, (2) simulated capture for 3 min to mimic the extended physical exercise during capture with rod and reel, (3) simulated capture with subsequent air exposure for 10 min to mimic immediate photography, (4) simulated capture and subsequent retention in a carp sack for 9 h to mimic the retention of carp overnight (for details on carp-sack retention of various durations, see Rapp et al. 2012), or (5) simulated capture, carp-sack retention, and subsequent air exposure to mimic the exposure of previously retained carp to air during photography. Each group comprised $n = 10$ fish (total $n = 100$ [50 for each temperature]; TL: 28.0 ± 2.6 cm, mean ± SD). Each individual carp was used only once during the experiment.

To generate data for the control groups for both water temperatures, a maximum of one fish per day was individually netted from a single holding tank to exclude the influence of sequential sampling on blood parameters (Pickering et al. 1982). Fish from all other treatment groups from both water temperatures were individually netted from a holding tank and transferred into a separate tank (200 L) where they were physically exhausted by tail pinching for 3 min, similar to the playing duration in the field experiment. Previous research has shown that this treatment results in similar physiological disruption as found in a real angling event (Ferguson and Tufts 1992). Subsequently, fish were exposed to air for 10 min to mimic realistic handling in specialized carp angling that includes weighing, measuring, and photography. Air exposure was conducted on a 90 × 50-cm unhooking mat (Daiwa, Groebenzell, Germany; model: Cormoran Standard Abhakmatte) typically used by specialized carp anglers to avoid dermal injuries of fish (Arlinghaus 2007). Fish were held in place on the unhooking mat by wet hands to prevent them from jumping or sliding off. Carp that were subjected to treatments that included retention or retention prior to air exposure were then transferred into carp sacks (Daiwa, Groebenzell, Germany; model: Cormoran Karpfensack "De Luxe"). The carp sacks were placed in a 900-L tank equipped with a filtration system and additionally supplied with 30 L tempered freshwater (at either 12°C or 22°C according to the temperature treatment) per hour. Carp were individually retained for 9 h. For the laboratory experiment, carp sacks were reduced in size to a dimension of 45 × 25 cm in consideration of the smaller body size of the carp used (Rapp et al. 2012).

Following each treatment, 6 mL of blood was collected by laterally inserted, caudal venipuncture (Dyer and Cervasio 2008) using heparinized syringes (B. Braun Melsungen AG, Melsungen, Germany; needle: 23 gauge, 31.8 mm; syringe: 1 mL; Omnifix-F1). Blood sampling was limited to 3 min, and fish that required more time were excluded from the study and replaced by a new individual. A total of 4 mL of whole blood was centrifuged (5,000 × g, 10 min), and plasma was removed. Plasma samples were immediately frozen in liquid nitrogen and later stored at −80°C for subsequent analyses. Plasma samples were analyzed for cortisol as a primary stress hormone, and for several indicators of the secondary stress response: glucose as an indicator of metabolic changes, plasma lactate as an indicator of anaerobiosis, and osmolality and plasma electrolytes (i.e., sodium, chloride, and potassium) as

indicators of osmotic and ionic disturbances (Rapp et al. 2012). The remaining 2 mL of whole blood were subdivided and used to assess blood pH (1 mL) and hematocrit (1 mL).

Field experiment.—The field experiment was conducted at Dow's Lake, a lentic section of the Rideau Canal, in Ottawa, Ontario (45°23′46.14″N, 75°42′03.09″W). Carp were captured on 18 fishing days between 9 September and 3 October 2007. Water temperature and dissolved oxygen were measured daily at 0800 hours when fishing took place and values ranged from 18.3°C to 23.1°C (20.2 ± 1.3°C, mean ± SD) and from 8.4 to 12.8 mg/L (10.4 ± 1.4 mg/L), respectively. All angling was conducted from shore at the same fishing site by one angler to exclude an influence of angler experience (Dunmall et al. 2001). Carp were captured with a gear type commonly used by specialized carp anglers. The terminal rig consisted of a fixed lead sinker and a short leader (for details see Rapp et al. 2008). This rig type facilitates shallow hooking, which reduces the potential for lethal injuries (Rapp et al. 2008). Shallow hooking also facilitates rapid hook removal to minimize the influence of additional handling and air exposure. Playing the fish during capture was standardized to 3 min similar to the laboratory experiment. Fish that were exhausted within a shorter time period were played in front of the shore until the time limit was reached. Fish that could not be landed within this time period were excluded from the study. Carp were landed with a knotless landing net to minimize dermal injuries (Barthel et al. 2003) and immediately transferred into a water-filled trough to avoid additional air exposure during unhooking and allow fish size to be recorded to the nearest 1 cm (TL: 68.5 ± 8.0 cm).

The experimental protocol of the field experiment equaled the laboratory experiment with the exception that as it was not feasible to include a minimally stressed control group as all capture methods cause stress in the fish, and wild fish as used in the field experiment may not react in the same manner when held in captivity as when kept in their natural environment (Cooke et al. 2002, 2013). However, the focal point of this study was to assess the relative difference among air exposure treatments and their respective nonair exposure treatments (i.e., capture versus air exposure, and capture and retention versus capture, retention, and air exposure). Fish that were assigned to the capture treatment group did not experience further handling, and blood samples were taken immediately after unhooking and measurement of TL. Fish that were subjected to an air exposure treatment following capture were placed on an unhooking mat and exposed to air for 10 min. For treatments that included retention or retention prior to air exposure, carp were individually transferred into carp sacks, which were used in their original dimension and measured 140 × 120 cm. Carp sacks were then placed into shallow water and attached to a metal pole on shore via an incorporated zip cord, and fish were left undisturbed for the entire 9-h retention period. Carp that were assigned to a combined retention and air exposure treatment were subsequently exposed to air for 10 min as previously described.

After completion of the respective treatments, fish were placed back in a water-filled trough to avoid additional air exposure and blood samples were taken by caudal venipuncture. A 3-mL sample of blood was withdrawn from each fish using Vacutainer syringes (Becton-Dickinson, Franklin Lakes, New Jersey; needle: 21 gauge, 38.1 mm; Vacutainer: 3 mL; lithium heparin anticoagulant). Duration of blood collection was limited to 1 min, and fish that required more time for sampling were excluded from the study. Plasma was separated by centrifugation (10,000 × *g*, 5 min), frozen in liquid nitrogen until the field sampling period was terminated, and then transferred to an ultracold freezer (−80°C). Plasma samples were analyzed for the same primary and secondary stress indicators as in the laboratory experiment with the exception of blood pH and hematocrit. In addition, LDH and AST were analyzed in the plasma as indicators of tissue cell damage

After blood sampling, fish remained in the water-filled trough and external transmitters (Holohil Systems, Carp, Ontario; model: PD-2 transmitters; weight in air: 3.8 g; battery life: 6 months; dimensions: 23 mm long × 12 mm wide × 7 mm high) were attached below the dorsal fin using the approach described by Cooke (2003) to study postrelease behavior. No anesthetic was used to avoid interference with postrelease behavior (Cooke et al. 2005). As the study was conducted in a public water body where carp were also targeted by local anglers, an additional anchor tag (Floy Manufacturing, Seattle, Washington) with a unique code and a phone number was inserted in the dorsal musculature. During the study period no carp was recaptured by the authors or other anglers.

Tracking was conducted from shore using handheld radio receivers (Lotek Wireless, Newmarket, Ontario; model: SRX 400) and three element Yagi antennas. Fish behavior was observed constantly for the first 30 min postrelease using successive gain reductions (zero-point tracking: Gravel and Cooke 2008; Gillis et al. 2010) to calculate distance moved within the first 30 min, time required to leave a predefined release site (area of 10 m around the specific release point) to assess time when fish resumed swimming postrelease, and time rested within 30 min postrelease (Rapp et al. 2012). Additional tracking points were taken from each fish at 1, 12, 24, 36, 48, 60, and 72 h postrelease using a combination of zero-point tracking and triangulation (Mech 1983) to calculate minimum displacement between successive locations (Rapp et al. 2012). Less frequent active tracking was conducted for a period of 2 months postrelease to assess long-term mortality. Varying transmitter locations between individual tracking dates indicated that fish were alive because predation on the large carp by any predator in the system was deemed impossible.

Biochemical blood analyses.—Plasma cortisol analysis was conducted with an enzyme-linked immunoabsorbent assay (ELISA) kit (IBL International GmbH, Hamburg, Germany; test: RE 52611) according to the manufacturer's protocol, and optical density was measured at 450 nm using a microplate reader (Tecan, Maennedorf, Switzerland; model: Spectrafluor

Plus). Plasma glucose from the laboratory experiment and plasma lactate were analyzed using standard assay kits according to the manufacturers' protocols (glucose: Diasys Diagnostic Systems GmbH, Holzheim, Germany; test: GOD FS. lactate: Trinity Biotech plc, Bray, Ireland; test: 735-10). Absorbance was measured at 500 nm for glucose and 540 nm for lactate using a plate reader (Tecan, Maennedorf, Switzerland; model: Genios). Blood pH was assessed potentiometrically with an open saltbridge microelectrode (Radiometer, Copenhagen, Denmark; model: BMS 2 Mk 2) connected to an acid–base analyzer (Radiometer, Copenhagen, Denmark; model: PHM 72). For hematocrit analysis well-mixed blood was drawn into microhematocrit tubes (Brand GmbH & Co. KG, Wertheim, Germany; size: 75×1.15 mm) and centrifuged ($12{,}000 \times g$, 10 min), and hematocrit was analyzed using a microhematocrit reader. For plasma osmolality analysis, a freezing-point osmometer was used (Gonotec GmbH, Berlin, Germany; model: Osmomat 030). All plasma ion analyses of the laboratory samples followed the protocols outlined in Zwirnmann et al. (1999).

Plasma cortisol, lactate, and osmolality from the field samples were analyzed as described above. Plasma glucose, sodium, chloride, potassium, LDH, and AST were analyzed using an autoanalyzer with appropriate reagents according to the manufacturer's protocol (Roche Hitachi, Basal, Switzerland; model: Roche/Hitachi 917). Analyses were based upon the International Federation of Clinical Chemistry and Laboratory Medicine Standard Reference Model.

Statistical analyses.—In the laboratory experiment, mean TL of fish did not differ significantly among treatment groups (two-way ANOVA: air exposure treatment factor: $F = 1.399$, df $= 4$, $P = 0.241$; water-temperature treatment factor: $F = 0.748$, df $= 1$, $P = 0.389$), and thus was not included in the subsequent analysis as a covariate. Instead, laboratory physiological variables were compared among treatments (four levels) and water temperature (two levels) using two-way ANOVA. Normality was tested using a Kolmogorov–Smirnov test ($P < 0.05$) and homogeneity of variance was evaluated using Levene's test ($P < 0.05$). If the basic assumptions were violated, data were subjected to a logarithmic transformation [$\ln(x + 1)$]. Transformation did not result in normal distribution for dependent variables; therefore, nontransformed data were used to compare the treatment groups with a two-way ANOVA due to the robustness to violations of the normality assumption (Zar 1999). In significant ANOVA models, a Tukey's post hoc test was used when the variances were homogeneous, and a Dunnett-T3 post hoc test when the variances were heterogeneous to assess which treatment groups differed from each other. In models with a significant temperature effect, a *t*-test was used to compare high and low water temperature levels within treatment groups, and *P*-values were adjusted by Bonferroni–Holm corrections for multiple comparisons (Holm 1979).

In the field experiment, plasma samples of five fish showed signs of hemolysis, icterus, or lipemia, which can result in analytical testing errors (Bellamy and Olexson 2000). Consequently, these fish were excluded from statistical analyses of physiological variables yielding lower sample sizes in some tests. All fish were included in the analyses of behavioral variables. Mean fish TL did not significantly differ among treatment groups (physiology, Kruskal–Wallis–H: $\chi^2 = 3.304$, df $= 3$, $P = 0.347$; behavior, one-way ANOVA: $F = 1.278$, df $= 3$, $P = 0.296$) and was therefore not included in the subsequent statistical analyses. Environmental variables (i.e., water temperature and dissolved oxygen) that could not be controlled in the field but may influence physiological variables, postrelease behavior, or survival of carp in response to the treatments did not differ significantly among treatment groups (all *P*-values $>$ 0.05 for each water variable during all investigations), indicating that carp in each treatment group experienced similar environmental conditions. Continuous variables (i.e., physiological variables, distance moved, time rested, and minimum displacement) were compared among treatment groups using one-way ANOVA after verifying that data met the assumptions for parametric tests. In case of deviations from underlying assumptions a logarithmic transformation [$\ln(x + 1)$] was applied, which resulted in normal distribution for some plasma (i.e., lactate and LDH) and behavioral (i.e., MDP from 31 to 60 min postrelease and MDP from 24 to 36 h postrelease) variables. In significant ANOVA models a Tukey's post hoc test was used when the variances were homogeneous, and a Dunnett-T3 post hoc test when the variances were heterogeneous to assess which treatment groups differed from each other. If the logarithmic transformation did not result in normal distribution (i.e., plasma cortisol, osmolality, chloride, and potassium), nontransformed data were used, and treatment groups were compared using a nonparametric Kruskal–Wallis–H test followed by Nemenyi post hoc tests (i.e., sample-size-independent Nemenyi post hoc tests for comparisons of physiological data, and sample-size-dependent Nemenyi post hoc tests for comparisons of behavioral data: Sachs and Hedderich 2006). The behavioral parameter "time required to leave the release site" was compared among treatment groups using Kaplan–Meier analyses with Breslow statistics for pairwise comparisons. The software package SPSS (SPSS, Chicago, Illinois; version 15.0) was used for all statistical analyses and significance was assessed at $P < 0.05$. All results presented in text, tables, and figures constitute nontransformed values to facilitate interpretation. Results are presented as mean $\pm$ SE.

RESULTS

Influence of Air Exposure on the Physiological Status of Common Carp in the Laboratory

All physiological variables related to the primary and secondary stress response were affected by treatment group or water temperature or the interaction of treatment groups and water temperature (Table 1). However, most differences causing significant overall ANOVA models were relative to the control group and resulted from simulated capture for 3 min or

TABLE 1. Test statistics of the two-way ANOVA on the influence of treatment and water temperature on physiological parameters of Common Carp in the laboratory experiments. Bold italics indicate significant effects ($P < 0.05$).

Variable	Type III sum of squares	F-value	df	P-value
Cortisol	66764.528	2.717	9	***0.008***
Treatment	51840.372	4.748	4	***0.002***
Water temperature	7261.918	2.660	1	0.106
Treatment × water temperature	7725.371	0.707	4	0.589
Glucose	236.381	9.017	9	***0.001***
Treatment	175.396	15.054	4	***0.001***
Water temperature	22.279	7.649	1	***0.007***
Treatment × water temperature	34.033	2.921	4	***0.026***
Lactate	925.114	47.298	9	***0.001***
Treatment	916.731	105.456	4	***0.001***
Water temperature	1.317	0.606	1	0.438
Treatment × water temperature	9.499	1.093	4	0.365
Blood pH	4.436	44.855	9	***0.001***
Treatment	4.329	98.481	4	***0.001***
Water temperature	0.057	5.187	1	***0.025***
Treatment × water temperature	0.071	1.608	4	0.179
Hematocrit	284.119	4.204	9	***0.001***
Treatment	158.196	5.266	4	***0.001***
Water temperature	113.666	15.136	1	***0.001***
Treatment × water temperature	12.030	0.400	4	0.808
Osmolality	6367.717	4.033	9	***0.001***
Treatment	4715.699	6.719	4	***0.001***
Water temperature	1374.002	7.831	1	***0.006***
Treatment × water temperature	240.178	0.342	4	0.849
Sodium	2147.252	1.895	9	0.063
Treatment	441.489	0.877	4	0.481
Water temperature	1598.136	12.697	1	***0.001***
Treatment × water temperature	37.903	0.075	4	0.990
Chloride	1719.639	2.094	9	***0.038***
Treatment	629.449	1.725	4	0.152
Water temperature	653.183	7.159	1	***0.009***
Treatment × water temperature	392.734	1.076	4	0.373
Potassium	50.531	14.648	9	***0.001***
Treatment	47.288	30.843	4	***0.001***
Water temperature	2.286	5.964	1	***0.017***
Treatment × water temperature	1.160	0.757	4	0.556

retention for 9 h and were not further influenced by air exposure (Table 2). This applied to plasma cortisol, which was elevated in retained carp compared with control fish, but neither 10 min air exposure after simulated capture nor air exposure after retention further increased the cortisol levels. Similarly, there was no statistically significant difference in plasma glucose between fish exposed to air after capture and fish captured only, as well as no significant difference between fish exposed to air following retention and fish that were only retained. Significant differences were between the air exposure treatment groups and the control group. However, plasma lactate and blood pH showed an additional effect of air exposure (Table 2). Plasma lactate increased during simulated capture relative to that of the control group, and a 24% increase was observed during air exposure relative to the capture treatment (Table 2). Plasma lactate levels of fish retained in carp sacks decreased to levels similar to those observed in control fish. Air exposure after retention, however, caused a plasma lactate increase of 358% relative to that in retained fish (Table 2). The blood pH dropped during capture relative to the control group, and a further decrease by 0.16 units was observed after carp were exposed to air. During retention, the blood pH increased above

TABLE 2. Blood biochemistry of Common Carp (mean ± SE) in the laboratory experiment. Treatment groups comprise control fish, carp subjected to exhaustive exercise of 3 min (simulated capture) without or with 10 min of air exposure, and carp subjected to simulated capture and retained in carp sacks for 9 h without or with 10 min of air exposure. Different lowercase letters indicate significant differences among treatment groups in the overall means across both water temperature levels ($P < 0.05$; for statistical results see Table 1). Bold italics indicate significant differences in the variable of interest between water temperatures within a given treatment ($P < 0.05$).

Variable	Control ($n = 10$)	Capture ($n = 10$)	Capture + air exposure ($n = 10$)	Capture + retention ($n = 10$)	Capture + retention + air exposure ($n = 10$)
Cortisol (ng/mL)					
22°C	46.3 ± 8.8	63.5 ± 10.4	84.7 ± 17.6	133.3 ± 25.5	88.8 ± 19.1
12°C	31.6 ± 12.2	68.3 ± 8.7	76.5 ± 21.9	85.0 ± 13.2	69.0 ± 21.2
Average	39.0 ± 7.5 z	66.1 ± 6.5 yz	80.6 ± 13.7 yz	109.2 ± 15.0 y	78.9 ± 14.0 yz
Glucose (mmol/L)					
22°C	***3.9 ± 0.4***	***5.5 ± 0.5***	***6.5 ± 0.6***	6.3 ± 0.4	6.6 ± 0.9
12°C	***2.5 ± 0.1***	***3.4 ± 0.1***	***4.3 ± 0.5***	7.0 ± 0.8	6.8 ± 0.6
Average	3.2 ± 0.3 z	4.4 ± 0.3 yz	5.4 ± 0.5 xy	6.7 ± 0.4 x	6.7 ± 0.6 x
Lactate (mmol/L)					
22°C	1.2 ± 0.1	7.4 ± 0.6	8.1 ± 0.4	1.0 ± 0.1	5.5 ± 0.7
12°C	1.5 ± 0.4	6.7 ± 0.6	9.3 ± 0.6	1.3 ± 0.2	5.6 ± 0.7
Average	1.3 ± 0.2 z	7.0 ± 0.4 x	8.7 ± 0.4 w	1.2 ± 0.1 z	5.5 ± 0.5 y
Blood pH					
22°C	7.59 ± 0.03	7.27 ± 0.03	7.13 ± 0.03	7.74 ± 0.03	7.29 ± 0.04
12°C	7.60 ± 0.03	7.35 ± 0.02	7.17 ± 0.02	7.72 ± 0.02	7.42 ± 0.06
Average	7.59 ± 0.02 x	7.31 ± 0.02 y	7.15 ± 0.02 z	7.73 ± 0.02 w	7.35 ± 0.04 y
Hematocrit (%)					
22°C	25.4 ± 0.9	26.2 ± 1.3	28.7 ± 0.7	26.0 ± 0.6	28.8 ± 0.9
12°C	22.9 ± 0.9	24.5 ± 1.0	26.5 ± 1.0	24.9 ± 0.5	25.6 ± 0.8
Average	24.2 ± 0.7 z	25.3 ± 0.8 yz	27.6 ± 0.6 y	25.5 ± 0.4 yz	27.2 ± 0.7 y
Osmolality (mOsmol/kg)					
22°C	272.4 ± 5.0	290.4 ± 3.7	288.5 ± 3.2	***277.3 ± 2.9***	290.1 ± 5.8
12°C	280.5 ± 4.6	292.6 ± 4.0	299.4 ± 3.3	***287.4 ± 1.7***	296.3 ± 6.8
Average	276.5 ± 3.4 z	291.7 ± 2.7 y	294.0 ± 2.6 y	282.4 ± 2.0 yz	293.2 ± 4.4 y
Sodium (mmol/L)					
22°C	134.0 ± 2.6	135.9 ± 5.4	130.2 ± 4.3	129.0 ± 4.2	133.6 ± 1.2
12°C	142.0 ± 5.3	143.4 ± 3.3	139.4 ± 2.1	138.6 ± 2.6	139.7 ± 2.0
Average	138.0 ± 3.0 z	140.0 ± 3.1 z	134.8 ± 2.6 z	133.8 ± 2.6 z	136.6 ± 1.3 z
Chloride (mmol/L)					
22°C	105.4 ± 1.4	100.9 ± 8.2	108.0 ± 1.0	100.5 ± 3.1	102.1 ± 2.7
12°C	106.8 ± 0.4	112.9 ± 2.0	112.3 ± 1.6	107.5 ± 1.5	103.3 ± 2.9
Average	106.1 ± 0.7 z	107.5 ± 4.0 z	110.2 ± 1.0 z	104.0 ± 1.9 z	102.7 ± 1.9 z
Potassium (mmol/L)					
22°C	***3.5 ± 0.2***	4.4 ± 0.2	4.2 ± 0.2	2.8 ± 0.2	2.9 ± 0.2
12°C	***2.8 ± 0.1***	4.2 ± 0.2	4.1 ± 0.2	2.4 ± 0.2	2.8 ± 0.2
Average	3.1 ± 0.1 z	4.3 ± 0.2 y	4.1 ± 0.2 y	2.6 ± 0.1 z	2.9 ± 0.1 z

control levels, and subsequent air exposure caused blood pH to decrease by 0.38 units and approach levels observed in carp that were exposed to simulated capture (Table 2). Hematocrit was not significantly affected by air exposure following capture or retention relative to the respective treatments without air exposure, and significance was based upon differences relative to the control group. Furthermore, air exposure did not result in ionic and osmotic disturbances relative to the respective treatments without air exposure, and differences in plasma osmolality and potassium levels were only relative to control fish (Table 2).

In addition to air exposure, water temperature had a significant effect on some physiological variables either individually as main effects (blood pH, hematocrit, osmolality, ions) or in interaction with treatments (glucose; Table 1). However, only a few pairwise comparisons among low (12°C) and high (22°C) temperature levels were ultimately found to be significantly

different (Table 2). In fact, plasma glucose was the only variable for which water temperature had a significant effect on the magnitude of physiological changes associated with the treatment (Table 1).

Influence of Air Exposure on Physiological Status and Tissue Damage of Common Carp in the Field

Similar to the laboratory experiment, several primary and secondary stress indicators and indicators of tissue damage differed significantly among treatment groups in the field, but only few differences were directly attributable to 10 min of air exposure (Table 3). In contrast to the laboratory experiment, exposing Common Carp to air for 3 min following capture in the field did not significantly increase plasma lactate concentrations relative to fish sampled immediately after capture. However, air exposure after 9 h of retention caused a plasma lactate increase of 89% compared with fish that were retained but not exposed to air, and the absolute plasma lactate level reached values similar to those observed following capture (Table 3). Plasma levels of cortisol, glucose, osmolality, sodium, chloride, potassium, LDH, and AST were not affected by air exposure (Table 3).

Influence of Air Exposure on the Postrelease Behavior and Survival of Common Carp in the Field

Although not statistically significant, air exposure for 10 min tended to decrease locomotor activity of Common Carp within the first 30 min postrelease relative to directly comparable treatment groups without air exposure as indicated by a P-value of 0.067 and most prominently in the fish exposed to air after retention in a carp sack for 9 h (Figure 1A). Additionally, air exposure following capture and retention significantly influenced how long released fish stayed at the release site (Figure 2). Thirty percent of fish exposed to air after capture and 50% of fish exposed to air following retention did not leave the release site within 30 min postrelease, while only 10% of the fish subjected solely to capture or retention remained at the release site

TABLE 3. Blood biochemistry of Common Carp (mean ± SE) in the field experiment. Treatment groups comprise fish captured for 3 min without or with 10 min of air exposure and carp captured and retained in carp sacks for 9 h without or with 10 min of air exposure. F-values refer to one-way ANOVA results, while χ^2-values refer to nonparametric Kruskal–Wallis–H test results. Different letters after values indicate significant differences between treatment groups ($P < 0.05$).

Variable	Capture ($n = 9$)	Capture + air exposure ($n = 9$)	Capture + retention ($n = 8$)	Capture + retention + air exposure ($n = 9$)	F-value or χ^2-value, df, P-value
Cortisol (ng/mL)	33.48 ± 24.93 z	59.69 ± 26.32 yz	188.68 ± 40.70 y	218.58 ± 40.04 y	$\chi^2 = 15.281$, df = 3, $P = 0.002$
Glucose (mmol/L)	4.23 ± 0.67 z	5.18 ± 0.79) z	8.64 ± 0.77 y	9.74 ± 0.39 y	$F = 15.971$, df = 3, $P < 0.001$
Lactate (mmol/L)	9.01 ± 1.08 y	10.08 ± 0.66 y	4.76 ± 0.88 z	9.01 ± 0.56 y	$F = 10.348$, df = 3, $P < 0.001$
Osmolality (mOsmol/kg)	318.56 ± 4.03	307.22 ± 8.97	311.00 ± 6.41	308.78 ± 7.13	$\chi^2 = 0.602$, df = 3, $P = 0.896$
Sodium (mmol/L)	158.33 ± 1.93 y	160.11 ± 2.35 y	149.88 ± 0.97 z	147.67 ± 2.08 z	$F = 10.072$, df = 3, $P < 0.001$
Chloride (mmol/L)	117.78 ± 1.02 y	116.22 ± 1.15 yz	116.00 ± 1.24 yz	109.89 ± 1.55 z	$\chi^2 = 14.337$, df = 3, $P = 0.002$
Potassium (mmol/L)	2.71 ± 0.36	3.48 ± 0.24	3.19 ± 0.26	2.72 ± 0.27	$\chi^2 = 5.043$, df = 3, $P = 0.169$
LDH (U/L)	382.00 ± 114.84	533.78 ± 221.94	1,087.13 ± 358.57	676.78 ± 179.84	$F = 2.533$, df = 3, $P = 0.075$
AST (U/L)	111.44 ± 17.05 z	128.78 ± 27.61 yz	230.00 ± 39.68 y	195.89 ± 33.27 yz	$F = 3.393$, df = 3, $P = 0.030$

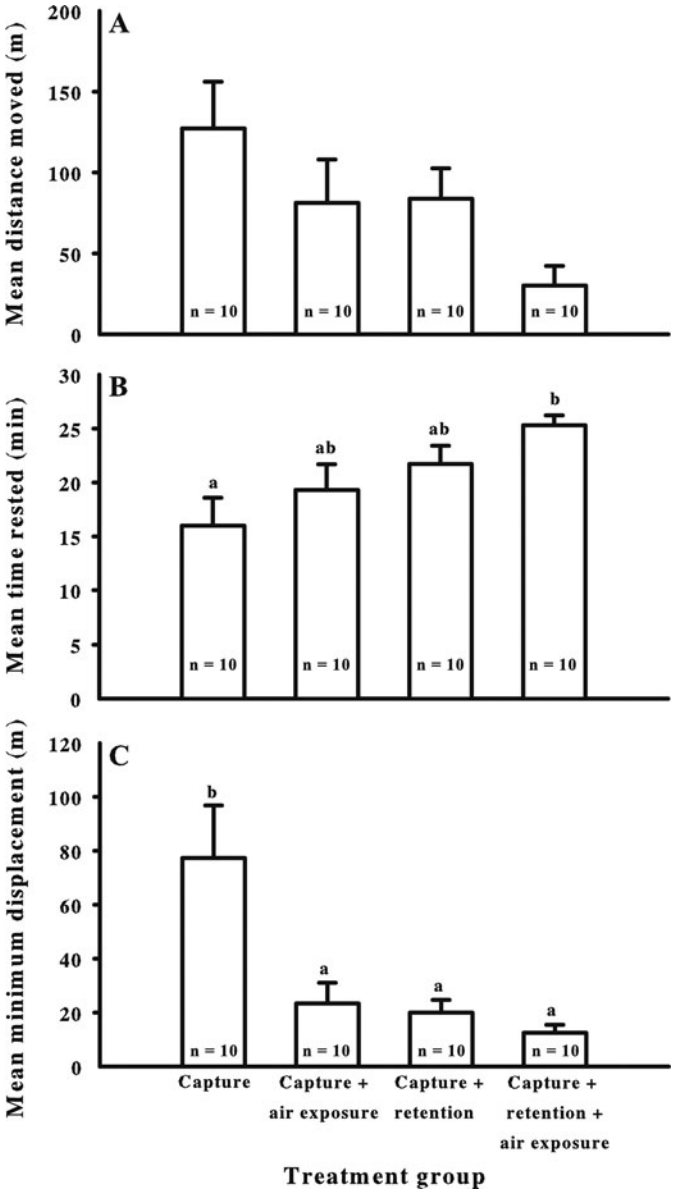

FIGURE 1. (**A**) Mean + SE distance moved (m) (one-way ANOVA: $F = 2.601$, df = 3, $P = 0.067$) and (**B**) mean + SE time rested (min) (one-way ANOVA: $F = 3.813$, df = 3, $P = 0.018$, Dunnett-T3 post hoc test) from 0 to 30 min postrelease in Common Carp captured without or with 10 min of air exposure, and carp captured and retained in carp sacks without or with 10 min of air exposure. (**C**) Mean + SE minimum displacement (m) (one-way ANOVA: $F = 5.244$, df = 3, $P = 0.004$, Tukey's post hoc test) between successive locations of carp from 31 to 60 min postrelease in carp captured for 3 min without or with 10 min of air exposure of, and carp captured and retained for 9 h in carp sacks without or with 10 min of air exposure. Different letters above bars indicate significant differences among treatment groups.

(Figure 2). By contrast, the length of time fish rested within the first 30 min postrelease did not differ significantly among air exposure and nonair exposure treatments, and significance of the ANOVA model was based upon a longer resting time between carp that were exposed to air following retention in a

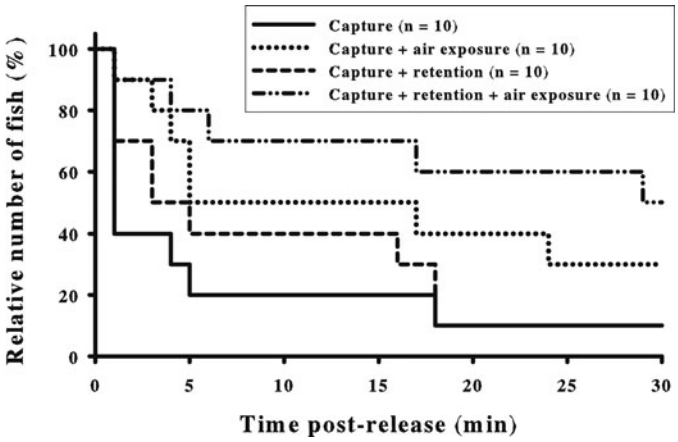

FIGURE 2. Relative numbers (%) of Common Carp at the release site plotted against the time after release (min) in carp captured without or with 10 min of air exposure, and carp captured and retained in carp sacks without or with 10 min of air exposure. Differences between treatment groups were significant (Kaplan–Meier: $\chi^2 = 9.46$, df = 3, $P = 0.024$).

carp sack and carp that were captured and immediately released (Figure 1B). From 31 to 60 min postrelease, minimum displacement was significantly reduced in fish exposed to air following capture relative to fish that were only captured. However, air exposure after retention did not further alter the already impaired movement activity of fish compared with retained fish that were not exposed to air (Figure 1C). Minimum displacement during subsequent tracking intervals from 12 to 72 h was not statistically different among treatment groups (Table 4). Air exposure after capture or retention in carp sacks did not result in any mortality during a postrelease observation period of 2 months.

DISCUSSION

Exposure to air and retention in carp sacks represent typical stressors in Common Carp recreational fisheries (Cooke and Suski 2005; Arlinghaus et al. 2007). Although the examined air exposure period of 10 min was long compared with what occurs in other fisheries, the additive effects of air exposure on the physiological stress response of carp were limited to plasma lactate and blood pH alterations after capture (laboratory) or retention in carp sacks (laboratory and field). Furthermore, the effect of temperature on the stress response was less pronounced than in other species (Gale et al. 2013). Despite few changes in physiological parameters, prolonged air exposure caused a tertiary stress response as revealed by decreased postrelease movement activity relative to fish that were not exposed to air, and fish required longer to disperse from the release site. These behavioral changes were, however, quickly reversed, and movement was similar between air-exposed and control fish by 12 h postrelease. Furthermore, no lethal consequences were observed. Our results overall confirmed the high resiliency of Common Carp to extended air exposure as it was previously reported in relation to other stressors (Rapp et al. 2012).

TABLE 4. Mean minimum displacement (MDP) ± SE (m) in Common Carp between successive locations from 12 to 72 h postrelease. Treatment groups comprise fish captured for 3 min without or with 10 min of air exposure and carp captured and retained in carp sacks without or with 10 min of air exposure. *F*-values refer to one-way ANOVA results. Differences between treatment groups were not significant ($P < 0.05$).

	Mean MDP (m)				
Time range postrelease (h)	Capture ($n = 10$)	Capture + air exposure ($n = 10$)	Capture + retention ($n = 10$)	Capture + retention + air exposure ($n = 10$)	*F*-value, df, *P*-value
1–12	346.57 ± 60.28	224.75 ± 52.70	278.62 ± 53.80	354.74 ± 33.94	$F = 1.489$, df = 3, $P = 0.238$
12–24	287.94 ± 91.60	371.04 ± 86.29	271.78 ± 48.26	184.01 ± 65.29	$F = 1.055$, df = 3, $P = 0.383$
24–36	220.61 ± 55.62	227.65 ± 49.65	305.36 ± 62.15	221.26 ± 92.61	$F = 1.403$, df = 3, $P = 0.263$
36–48	158.65 ± 27.03	191.25 ± 47.43	248.36 ± 42.81	192.40 ± 55.30	$F = 0.690$, df = 3, $P = 0.565$
48–60	306.09 ± 65.44	131.08 ± 41.89	201.90 ± 65.62	212.32 ± 52.53	$F = 1.565$, df = 3, $P = 0.218$
60–72	265.14 ± 59.26	176.24 ± 57.38	201.76 ± 77.46	170.36 ± 40.59	$F = 0.521$, df = 3, $P = 0.671$

The lack of a clear plasma cortisol response between air exposure treatments and the treatments without air exposure may be attributed to the timing of blood sampling, which was conducted immediately after 10 min of air exposure. Cortisol typically peaks in the blood stream with a lag time of up to a few hours after the initial stress experience (e.g., Pickering et al. 1982; Davis and Schreck 2005; Haukenes and Buck 2006). For example, Dabrowska et al. (1991) observed increased cortisol levels in carp that were exposed to air for 30 s when blood samples were taken 1 h after the treatment. Thus, it may be possible that we underestimated the impact of air exposure on plasma cortisol as this physiological variable may have continued to rise after blood sampling (Cooke et al. 2013). Although qualitatively similar (i.e., no significant additional effect of air exposure), plasma cortisol levels differed quantitatively between the laboratory and the field experiment. Cortisol levels in carp in the laboratory after simulated capture and capture with subsequent air exposure were higher than those observed in the field, while levels following retention and retention and subsequent air exposure were lower in the laboratory fish compared with those in the field experiment. Quantitative differences in the neuroendocrine stress response are frequently observed as a result of various extrinsic and intrinsic factors (Barton et al. 2002). The observed patterns may, for example, be partially attributable to a difference between the two fish groups in the stress response resulting from carp-sack retention; we used hatchery fish in the laboratory but used feral carp in the field experiment. In addition, the size of fish differed between the laboratory and the field experiments, which may have affected their stress response (Ferguson et al. 1993; Kieffer et al. 1996; Gingerich and Suski 2012).

Air exposure following capture or retention did not significantly increase plasma glucose in carp relative to the nonair exposure treatments in both the laboratory and the field experiment. Similar to plasma cortisol, this may be attributed to immediate blood sampling following the treatment. Previous studies have reported a lag time between exposure to a stressor and a glucose peak in the blood stream (Pickering et al. 1982; Davis and Schreck 2005; Haukenes and Buck 2006). Hyperglycemia is a frequent response reflecting increased energy demands during stress and is caused by hormonal stimulation of glycogenolysis and gluconeogenesis (Wendelaar Bonga 1997; Barton et al. 2002). The delayed glucose appearance in the blood stream may be caused by lag time between hormonal stimulation of these pathways and the subsequent diffusion of glucose into the general circulation (Haukenes and Buck 2006).

In the laboratory experiment, carp that were exposed to air following simulated capture had significantly higher plasma lactate levels compared to fish that were captured only, which is consistent with studies in other species (Ferguson and Tufts 1992; Haukenes and Buck 2006; Arlinghaus et al. 2009). In the field experiment, lactate moderately increased during air exposure after capture relative to the capture treatment, although

differences were not significant. Mean plasma lactate levels in carp in the laboratory and in the field were lower than those approached in other studies with Common Carp subjected to hypoxia (13.84 mmol/L: Van Raaij et al. 1996) or capture and subsequent retention a carp sack (laboratory, 9.5 mmol/L; field, 12.0 mmol/L: Rapp et al. 2012). Lactate accumulates as the end product of anaerobic consumption of the endogenous fuels adenosine triphosphate (ATP), phosphocreatine, and glycogen. Previous studies showed that the release of lactate from the muscle into the blood stream is slow, which can result in a lag time of up to few hours between exposure to the stressor and maximum blood lactate levels (Pickering et al. 1982; Haukenes and Buck 2006). Similarly, Rapp et al. (2012) observed the plasma lactate peak following capture after a brief retention period of 0.5 h and 3 h in a laboratory and field experiment with carp, respectively, which may indicate that maximum levels were not approached immediately after the air exposure treatment when blood samples were taken. During retention in carp sacks, plasma lactate in carp decreased in the laboratory and approached control levels. Similarly, plasma lactate levels observed following retention of carp in the field were lower than levels observed after capture. In both experiments, subsequent air exposure resulted in a pronounced increase in plasma lactate, which is in agreement with studies that examined the effect of air exposure on plasma lactate following retention in Largemouth Bass (Suski et al. 2004) and Walleye (Killen et al. 2006). In both the laboratory and the field, the incremental plasma lactate increase following retention (laboratory: 358%, field: 89%) was more pronounced than the incremental plasma increase during air exposure following capture (laboratory: 24%, field: 12%), which could be related to physical recovery of the carp during retention. Previous research has demonstrated that recovered fish show higher activity compared with fish that are exhausted from previous capture (Brownscombe et al. 2013). Increased activity following retention in a carp sack can contribute to the rather lengthy air exposure period in specialized carp angling as it includes a time period until body movements of carp cease sufficiently to enable anglers to photograph a large fish, and it may increase the consumption of endogenous energy fuels compared with fish that are resting during air exposure (White et al. 2008). Thus, the reduced movement during air exposure of fish already exhausted following capture might have contributed to the lower incremental plasma lactate increase compared with the higher plasma lactate increase observed in fish following retention (White et al. 2008).

The blood pH decreased during simulated capture and dropped further during subsequent air exposure resulting in a more severe extracellular acidosis than caused by capture alone, which was similarly observed by Ferguson and Tufts (1992) in Rainbow Trout *Oncorhynchus mykiss*. The acidosis caused by simulated capture may be of mixed metabolic and respiratory origin due to association and dissociation of lactate and subsequent proton leakage into the blood stream and increased CO_2 in the blood, as this has been observed in other studies that exposed fish to forced exercise (Wood 1991; Ferguson and Tufts 1992). By contrast, the drop in blood pH during air exposure may largely be caused by the inhibition of gas exchange (Boutilier 1990; Ferguson and Tufts 1992). During recovery from simulated capture in carp sacks, blood pH increased resulting in a slight alkalosis possibly of metabolic origin (Milligan and Wood 1986; Wood 1991), and subsequent air exposure again caused a blood pH drop to levels similar to those observed after simulated capture.

Water temperature affected few physiological variables of Common Carp in our study, but interactive effects among air exposure treatments and water temperature were only significant for plasma glucose. Effects of air exposure on plasma glucose were exacerbated at high water temperature, which may be a result of increased metabolism at higher water temperatures (Barton et al. 2002). The resilience of carp to air exposure at 22°C contrasts with studies that found increased sublethal and lethal consequences of air exposure at elevated water temperatures (reviewed in Gale et al. 2013). Most of the available research studied stenothermic coldwater fishes (e.g., various salmonid species), which are sensitive to elevated water temperatures beyond certain thresholds. Potentially, water temperatures tested here did not exceed a threshold in eurythermic Common Carp, which has a preference for warm water temperatures (Blanck et al. 2007) and an overall wide temperature tolerance range of 4–35°C (Plumb and Hanson 2010). Pörtner (2001, 2002) suggested that temperature tolerance of fish is linked to the performance of the oxygen supply chain, and several studies revealed that Common Carp are able to maintain aerobic metabolism over a wide temperature range of at least 5–25°C by increasing respiratory and cardiovascular functions at higher temperatures (Ott et al. 1980; Glass et al. 1990; Stecyk and Farrell 2006). Thus, it seems likely that the high water temperature of 22°C tested in this study did not exceed an upper threshold at which aerobic ATP generation is limited due to increased metabolic rate and thus did not reduce aerobic scope (Pörtner 2001, 2002). Furthermore, it seems likely that the low water temperature of 12°C tested did not exceed a lower threshold at which limited muscular performance causes a decrease in external (gill) and internal (heart) ventilatory capacity and similarly a decrease in aerobic scope (Pörtner 2002). The fact that temperature treatments were moderate for the carp coupled with the general resilience of Common Carp to hypoxia (Lykkeboe and Weber 1978; Van den Thillart and Van Waarde 1991) may explain the lack of a compounding temperature effect.

Despite minor levels of physiological disturbances and no evidence of increased tissue damage in air-exposed carp in the field setting, postrelease movement activity was impaired as revealed by differences in the dispersal pattern of fish from the release site. In some cases, we qualitatively observed equilibrium loss in both treatment groups involving air exposure, which was previously observed in other species that were subjected to extended air exposure periods (Cooke and Philipp 2004; Danylchuk et al. 2007; Gingerich et al. 2007). However, visual observation of

equilibrium loss was only possible for fish that rested directly at the release point, but equilibrium loss can occur after fish leave the release site (Danylchuk et al. 2007). Minimum displacement within the first hour postrelease tended to be reduced in fish exposed to air following capture relative to fish that were captured but not exposed to air, although no significant differences in physiological variables in carp between these treatments were observed. The lack of differences in minimum displacement may be partly attributed to a temporally delayed response of some plasma variables as explained above, although other authors have noted a lack of concordance between physiological responses to stress and various organismal endpoints in a catch-and-release context (Davis and Schreck 2005; Arlinghaus et al. 2009; Cooke et al. 2013). In fact, swimming activity involves a multitude of biochemical and physiological processes, and it is very likely that the observed behavioral changes reflect disruption of additional physiological processes (Schreck et al. 1997; Little 2002; Cooke et al. 2013). Thus, the behavioral changes shown by carp exposed to air may be interpreted as an integrative response of various physiological changes stemming from air exposure and related stressors. Although not significantly different, carp that were exposed to air following retention tended to swim less compared with fish that were retained in carp sacks, and swam the least of all treatment groups, which may be a result of additive effects of retention stress and anaerobiosis.

Other studies have reported a positive correlation between the duration of air exposure and the magnitude of behavioral changes (Cooke and Philipp 2004; Schreer et al. 2005; Arlinghaus et al. 2009). We were unable to test a gradient of air exposure duration and instead exposed carp to air for 10 min, as this represents a realistic air exposure period in specialized Common Carp angling, which involves measuring, weighing, and photographing a large carp. It is thus unknown how different air exposure periods would influence carp behavior, and which durations can be regarded as critical thresholds as documented in other species (Schreer et al. 2005; Arlinghaus et al. 2009). Consistent with other studies reporting rapid behavioral recovery from catch-and-release angling stressors (Arlinghaus et al. 2009; Rapp et al. 2012), movement alterations seemed to be reversed by 12 h postrelease as subsequent tracking intervals revealed no differences in minimum displacement among treatment groups. However, potentially longer-lasting behavioral alterations (e.g., angling vulnerability: Beukema 1970; Raat 1985; Klefoth et al. 2013; habitat selection: Klefoth et al. 2011) in carp after specialized angling activities compared with fish in a pre-angled state remain unknown.

During the 2-month postrelease observation period no mortalities occurred, whereas several authors have noted direct (Ferguson and Tufts 1992; Gingerich et al. 2007) and indirect mortalities (Cooke and Philipp 2004; Danylchuk et al. 2007) in fish subjected to air exposure for shorter time periods after simulated capture. The resilience of carp to direct mortalities may be due to species-specific characteristics as Common Carp are very tolerant to low oxygen levels and are capable of coping with severe hypoxia or even anoxic conditions for short time periods (Lykkeboe and Weber 1978; Van den Thillart and Van Waarde 1991). Observed indirect mortalities in previous studies were facilitated by impaired postrelease behavior in conjunction with equilibrium loss and high predator abundance, which increases the susceptibility of released fish to predation (Cooke and Philipp 2004; Danylchuk et al. 2007). Large Common Carp, however, have reached a size most likely to be avoided by other predatory freshwater fishes, although smaller carp have been found in the diets of European otter *Lutra lutra* (Britton et al. 2005). Thus, it cannot be assured that angling would not increase mortality in areas with established piscivorous mammal populations as postrelease predation rates are highly dependent on predator occurrence (Cooke and Philipp 2004). However, the high resilience of Common Carp to hypoxic and even anoxic conditions and the large body size of carp render these fish unlikely to suffer mortality from 10 min of air exposure alone under most realistic angling conditions.

Two divergent conclusions can be drawn from this study depending on the individual perspective. From a management perspective that is interested in survival of individual fish as an endpoint, the impacts of air exposure during photography following capture or retention and other handling practices (e.g., measuring and weighing) are of minor concern in Common Carp. These findings may be of particular interest for commercially managed water bodies (i.e., fee-fishing operations), which are gaining popularity in Europe and North America and are dependent on angler satisfaction to make profits (Arlinghaus and Mehner 2003; Masser and Higginbotham 2012). Fee-fishing operations are often stocked with trophy specimens, which are of high value, and their death could decrease future income as the chance to catch an exceptional specimen attracts specialized carp anglers (Arlinghaus and Mehner 2003; Masser and Higginbotham 2012). The resilience of Common Carp towards handling practices associated with air exposure allows managers to develop regulations that meet the demands of specialized carp anglers (e.g., permission of photography after capture or retention in a carp sack) without significant mortalities and thus potential loss of revenue. By contrast, if one adopts an individual-based fish welfare perspective that attempts to minimize all forms of physiological or behavioral changes (Arlinghaus et al. 2007), the results indicate that anglers may limit impacts on carp by carrying out a quick photography session after capture or only briefly expose fish to air while keeping gills submerged. Furthermore, photography should be followed by the immediate release of the fish because Rapp et al. (2012) demonstrated that carp showed a physiological and behavioral stress response towards retention, and the present study showed that there are synergistic effects of retention and air exposure. We caution that this study was conducted at rather moderate water temperatures for Common Carp and even more care should be taken to minimize air exposure when water temperatures are more extreme.

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REFERENCES

Arlinghaus, R. 2007. Voluntary catch-and-release can generate conflict within the recreational angling community: a qualitative case study of specialized carp, *Cyprinus carpio*, angling in Germany. Fisheries Management and Ecology 14:161–171.

Arlinghaus, R., S. J. Cooke, J. Lyman, D. Policansky, A. Schwab, C. Suski, S. G. Sutton, and E. B. Thorstad. 2007. Understanding the complexity of catch-and-release in recreational fishing: an integrative synthesis of global knowledge from historical, ethical, social, and biological perspectives. Reviews in Fisheries Science 15:75–167.

Arlinghaus, R., T. Klefoth, S. J. Cooke, A. Gingerich, and C. Suski. 2009. Physiological and behavioral consequences of catch-and-release angling on Northern Pike (*Esox lucius* L.). Fisheries Research 97:223–233.

Arlinghaus, R., and T. Mehner. 2003. Socio-economic characterisation of specialized Common Carp (*Cyprinus carpio* L.) anglers in Germany, and implications for inland fisheries management and eutrophication control. Fisheries Research 61:19–33.

Barthel, B. L., S. J. Cooke, C. D. Suski, and D. P. Philipp. 2003. Effects of landing net mesh type on injury and mortality in a freshwater recreational fishery. Fisheries Research 63:275–282.

Barton, B. A., J. D. Morgan, and M. M. Vijayan. 2002. Physiological and condition-related indicators of environmental stress in fish. Pages 111–148 *in* S. M. Adams, editor. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland.

Bellamy, J. E. C., and D. W. Olexson. 2000. Quality assurance handbook for veterinary laboratories. Iowa State University Press, Ames.

Beukema, J. J. 1970. Angling experiments with carp (*Cyprinus carpio* L). II. Decreasing catchability through one-trial learning. Netherlands Journal of Zoology 20:81–92.

Blanck, A., P. A. Tedesco, and N. Lamouroux. 2007. Relationships between life-history strategies of European freshwater fish species and their habitat preferences. Freshwater Biology 52:843–859.

Boutilier, R. G. 1990. Control and co-ordination of gas exchange in bimodal breathers. Pages 280–346 *in* R. G. Boutilier, editor. Advances in comparative and environmental physiology 6: vertebrate gas exchange: from environment to cell. Springer-Verlag, Berlin.

Britton, J. R., J. S. Shepherd, S. Toms, and V. Simpson. 2005. Presence of carp, *Cyprinus carpio*, in the diet of the otter, *Lutra lutra*. Fisheries Management and Ecology 12:221–223.

Brownscombe, J. W., J. D. Thiem, C. Hatry, F. Cull, C. R. Haak, A. J. Danylchuk, and S. J. Cooke. 2013. Recovery bags reduce post-release impairments in locomotory activity and behaviour of bonefish (*Albula* spp.) following exposure to angling-related stressors. Journal of Experimental Marine Biology and Ecology 440:207–215.

Butcher, P. A., M. K. Broadhurst, K. C. Hall, and S. J. Cooke. 2011. Post-release survival and physiology of angled Luderick (*Girella tricuspidata*) after confinement in keeper nets in an Australian estuary. ICES Journal of Marine Science 68:572–579.

Cooke, S. J. 2003. Externally attached radio transmitters do not affect the parental care behavior of Rock Bass. Journal of Fish Biology 62:965–970.

Cooke, S. J., and D. P. Philipp. 2004. Behavior and mortality of caught-and-released bonefish (*Albula* spp.) in Bahamian waters with implications for a sustainable recreational fishery. Biological Conservation 118:599–607.

Cooke, S. J., and C. D. Suski. 2005. Do we need species-specific guidelines for catch-and-release recreational angling to effectively conserve diverse fishery resources? Biodiversity and Conservation 14:1195–1209.

Cooke, S. J., J. F. Schreer, K. M. Dunmall, and D. P. Philipp. 2002. Strategies for quantifying sub-lethal effects of marine catch-and-release angling: insights from novel freshwater applications. Pages 121–134 *in* J. A. Lucy and A. L. Studholme, editors. Catch and release in marine recreational fisheries. American Fisheries Society, Symposium 30, Bethesda.

Cooke, S. J., G. T. Crossin, D. A. Patterson, K. K. English, S. G. Hinch, J. L. Young, R. F. Alexander, M. C. Healey, G. Van Der Kraak, and A. P. Farrell. 2005. Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behavior and survivorship of Sockeye Salmon: development and validation of a technique. Journal of Fish Biology 67:1342–1358.

Cooke, S. J., M. R. Donaldson, C. M. O'Connor, G. D. Raby, R. Arlinghaus, A. J. Danylchuk, K. C. Hanson, S. G. Hinch, T. D. Clark, D. A. Patterson, and C. D. Suski. 2013. The physiological consequences of catch-and-release angling: perspectives on experimental design, interpretation, extrapolation, and relevance to stakeholders. Fisheries Management and Ecology 20:268–287.

Dabrowska, H., K. Dabrowski, K. Meyer-Burgdorff, W. Hanke, and K.-D. Gunther. 1991. The effect of large doses of vitamin C and magnesium on stress responses in Common Carp, *Cyprinus carpio*. Comparative Biochemistry and Physiology A 99:681–685.

Danylchuk, S. E., A. J. Danylchuk, S. J. Cooke, T. L. Goldberg, J. Koppelman, and D. P. Philipp. 2007. Effects of recreational angling on the post-release behavior and predation of Bonefish (*Albula vulpes*): the role of equilibrium status at the time of release. Journal of Experimental Marine Biology and Ecology 346:127–133.

Davis, M. W., and C. B. Schreck. 2005. Responses by pacific halibut to air exposure: lack of correspondence among plasma constituents and mortality. Transactions of the American Fisheries Society 134:991–998.

Dunmall, K. M., S. J. Cooke, J. F. Schreer, and R. S. McKinley. 2001. The effect of scented lures on the hooking injury and mortality of Smallmouth Bass caught by novice and experiences anglers. North American Journal of Fisheries Management 21:242–248.

Dyer, S. M., and E. L. Cervasio. 2008. An overview of restraint and blood collection techniques in exotic pet practice. Veterinary Clinics of North America: Exotic Animal Practice 11:423–443.

Farooqi, M. 2006. The world according to carp. Texas Park and Wildlife Magazine, 3. Available: http://www.tpwmagazine.com/archive/2006/mar/ed_3/. (August 2013).

Ferguson, R. A., J. D. Kieffer, and B. L. Tufts. 1993. The effects of body size on the acid-base and metabolite status in the white muscle of Rainbow Trout before and after exhaustive exercise. Journal of Experimental Biology 180:195–207.

Ferguson, R. A., and B. L. Tufts. 1992. Physiological effects of brief air exposure in exhaustively exercised Rainbow Trout (*Oncorhynchus mykiss*): implications for "catch and release" fisheries. Canadian Journal of Fisheries and Aquatic Sciences 49:1157–1162.

Gale, M. K., S. G. Hinch, and M. R. Donaldson. 2013. The role of temperature in the capture and release of fish. Fish and Fisheries 14:1–33.

Gillis, N. C., T. Rapp, C. T. Hasler, H. Wachelka, and S. J. Cooke. 2010. Spatial ecology of adult Muskellunge (*Esox masquinongy*) in the urban Ottawa reach of the historic Rideau Canal, Canada. Aquatic Living Resources 23:225–230.

Gingerich, A. J., S. J. Cooke, K. C. Hanson, M. R. Donaldson, C. T. Hasler, C. D. Suski, and R. Arlinghaus. 2007. Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. Fisheries Research 86:169–178.

Gingerich, A. J., and C. D. Suski. 2012. The effect of body size on post-exercise physiology in Largemouth Bass. Fish Physiology and Biochemistry 38:329–340.

Glass, M. L., N. A. Andersen, M. Kruhoffer, E. M. Williams, and N. Heisler. 1990. Combined effects of environmental P_{O2} and temperature on ventilation and blood gases in the carp *Cyprinus carpio* L. Journal of Experimental Biology 148:1–17.

Gravel, M.-A., and S. J. Cooke. 2008. Severity of barotrauma influences the physiological status, postrelease behavior, and fate of tournament-caught Smallmouth Bass. North American Journal of Fisheries Management 28:607–617.

Haukenes, A. H., and C. L. Buck. 2006. Time course of osmoregulatory, metabolic, and endocrine stress responses of Pacific Halibut following a 30-min air exposure. Journal of Applied Ichthyology 22:382–387.

Henry, J. B. 1996. Clinical diagnosis and management by laboratory methods. W. B. Saunders, Philadelphia.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:65–70.

Huntingford, F. A., C. Adams, V. A. Braithwaite, S. Kadri, T. G. Pottinger, P. Sandøe, and J. F. Turnbull. 2006. Current issues in fish welfare. Journal of Fish Biology 68:332–373.

Kieffer, J. D., R. A. Ferguson, J. E. Tompa, and B. L. Tufts. 1996. Relationship between body size and anaerobic metabolism in Brook Trout and Largemouth Bass. Transactions of the American Fisheries Society 125:760–767.

Killen, S. S., C. D. Suski, S. J. Cooke, D. P. Philipp, and B. L. Tufts. 2006. Factors contributing to the physiological disturbance in Walleyes during simulated live-release angling tournaments. Transactions of the American Fisheries Society 135:557–569.

Killen, S. S., C. D. Suski, M. B. Morrissey, P. Dyment, M. Furimsky, and B. L. Tufts. 2003. Physiological responses of Walleyes to live-release angling tournaments. North American Journal of Fisheries Management 23:1238–1246.

Klefoth, T., A. Kobler, and R. Arlinghaus. 2011. Behavioural and fitness consequences of direct and indirect non-lethal disturbances in a catch-and-release Northern Pike (*Esox lucius*) fishery. Knowledge and Management of Aquatic Ecosystems 403:11p1–11p18.

Klefoth, T., T. Pieterek, and R. Arlinghaus. 2013. Impacts of domestication on angling vulnerability of Common Carp, *Cyprinus carpio*: the role of learning, foraging behaviour and food preferences. Fisheries Management and Ecology 20:174–186.

Little, E. E. 2002. Behavioral measures of environmental stressors in fish. Pages 431–472 *in* S. M. Adams, editor. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland.

Lykkeboe, G., and R. E. Weber. 1978. Changes in respiratory properties of blood in the carp, *Cyprinus carpio*, induced by diurnal variation in ambient oxygen tension. Journal of Comparative Physiology 128:117–125.

Masser, M. P., and B. Higginbotham. 2012. Fee-fishing opportunities. Pages 329–349 *in* J. W. Neal, and D. W. Willis, editors. Small impoundment management in North America. American Fisheries Society, Bethesda, Maryland.

Mech, L. D. 1983. Handbook of animal radio-tracking. University of Minnesota Press, Minneapolis.

Milligan, C. L., and C. M. Wood. 1986. Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the Rainbow Trout. Journal of Experimental Biology 123:91–121.

Morrissey, M. B., C. D. Suski, K. R. Esseltine, and B. L. Tufts. 2005. Incidence and physiological consequences of decompression in Smallmouth Bass after live-release angling tournaments. Transactions of the American Fisheries Society 134:1038–1047.

Ott, M. E., N. Heisler, and G. R. Ultsch. 1980. A re-evaluation of the relationship between temperature and the critical oxygen-tension in freshwater fishes. Comparative Biochemistry and Physiology A 67:337–340.

Pickering, A. D., T. G. Pottinger, and P. Christie. 1982. Recovery of the Brown Trout, *Salmo trutta* L., from acute handling stress: a time-course study. Journal of Fish Biology 20:229–244.

Plumb, J. A., and L. A. Hanson. 2010. Health maintenance and principal microbial diseases of cultured fishes, 3rd edition. Wiley-Blackwell Scientific Publications, Ames, Iowa.

Pörtner, H. O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137–146.

Pörtner, H. O. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comparative Biochemistry and Physiology A 132:739–761.

Pottinger, T. G. 1998. Changes in blood cortisol, glucose and lactate in carp retained in anglers' keepnets. Journal of Fish Biology 53:728–742.

Quade, H. W. 2009. The multifacted carp. Blue Earth County Historical Society, Mankato, Minnesota.

Raat, A. J. P. 1985. Analysis of angling vulnerability of Common Carp, *Cyprinus carpio* L., in catch-and-release angling in ponds. Aquaculture and Fisheries Management 16:171–187.

Rapp, T., S. J. Cooke, and R. Arlinghaus. 2008. Exploitation of specialised fisheries resources: the importance of hook size in recreational angling for large Common Carp (*Cyprinus carpio* L.). Fisheries Research 94:79–83.

Rapp, T., J. Hallermann, S. J. Cooke, S. K. Hetz, S. Wuertz, and R. Arlinghaus. 2012. Physiological and behavioral consequences of capture and retention in carp sacks on Common Carp (*Cyprinus carpio* L.), with implications for catch-and-release recreational fishing. Fisheries Research 125–126:57–68.

Sachs, L., and J. Hedderich. 2006. Angewandte statistik: methodensammlung mit R. [Applied statistics: methods collection with R.] Springer-Verlag, Berlin.

Schreck, C. B., B. L. Olla, and M. W. Davis. 1997. Behavioral responses to stress. Pages 145–170 *in* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. P. Schreck, editors. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, UK.

Schreer, J. F., D. M. Resch, M. L. Gately, and S. J. Cooke. 2005. Swimming performance of brook trout after simulated catch-and-release angling: looking for air exposure thresholds. North American Journal of Fisheries Management 25:1513–1517.

Stecyk, J. A. W., and A. P. Farrell. 2006. Regulation of the cardiorespiratory system of Common Carp (*Cyprinus carpio*) during severe hypoxia at three seasonal acclimation temperatures. Physiological and Biochemical Zoology 79:614–627.

Suski, C. D., S. S. Killen, S. J. Cooke, J. D. Kieffer, D. P. Philipp, and B. L. Tufts. 2004. Physiological significance of the weigh-in during live-release angling tournaments for Largemouth Bass. Transactions of the American Fisheries Society 133:1291–1303.

Suski, C. D., S. S. Killen, M. B. Morrissey, S. G. Lund, and B. L. Tufts. 2003. Physiological changes in Largemouth Bass caused by live-release angling tournaments in southeastern Ontario. North American Journal of Fisheries Management 23:760–769.

Thompson, L. A., S. J. Cooke, M. R. Donaldson, K. C. Hanson, A. Gingerich, T. Klefoth, and R. Arlinghaus. 2008. Physiology, behavior, and survival of angled and air-exposed Largemouth Bass. North American Journal of Fisheries Management 28:1059–1068.

Van den Thillart, G., and A. Van Waarde. 1991. pH changes in fish during environmental anoxia and recovery: the advantages of the ethanol pathway. Pages 173–190 *in* A. J. Woakes, M. K. Grieshaber, and C. R. Bridges, editors. Physiological Ssrategies for gas exchange and metabolism. Cambridge University Press, Cambridge, UK.

Van Raaij, M. T. M., G. E. E. J. M. Van den Thillart, G. J. Vianen, D. S. S. Pit, P. H. M. Balm, and A. B. Steffens. 1996. Substrate mobilization and hormonal changes in Rainbow Trout (*Oncorhynchus mykiss*, L.) and Common Carp (*Cyprinus carpio*, L.) during deep hypoxia and subsequent recovery. Journal of Comparative Physiology B 166:443–452.

Wendelaar Bonga, S. E. 1997. The stress response in fish. Physiological Reviews 77:591–625.

White, A. J., J. F. Schreer, and S. J. Cooke. 2008. Behavioral and physiological responses of the congeneric Largemouth (*Micropterus salmoides*) and Smallmouth Bass (*M. dolomieu*) to various exercise and air exposure durations. Fisheries Research 89:9–16.

Wood, C. M. 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. Journal of Experimental Biology 160:285–308.

Zar, J. H. 1999. Biostatistical analysis, 4th edition. Prentice-Hall, Englewood Cliffs, New Jersey.

Zwirnmann, E., A. Krüger, and J. Gelbrecht. 1999. Analytik im zentralen chemielabor des IGB. [Analytical techniques of the central chemical laboratory of the IGB.] Berichte des IGB 9:3–24.