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# Ship noise and cortisol secretion in European freshwater fishes

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## ABSTRACT

Underwater noise pollution is a growing problem in aquatic environments and as such may be a major source of stress for fish. In the present study, we addressed the effects of ship noise and continuous Gaussian noise on adrenal activity in three European freshwater species. Underwater ship noise recorded in the Danube River and two Austrian lakes was played back to fish at levels encountered in the field (153 dB re 1  $\mu$ Pa, 30 min). Post exposure cortisol secretion was compared with control situations. Cortisol was measured with enzyme immunoassay techniques (EIA, ng cortisol/l water/g fish) in extracted aquarium water with corrections for fish mass. In the first series, two hearing specialists, the common carp (*Cyprinus carpio*) and the gudgeon (*Gobio gobio*) and one hearing generalist, the European perch (*Perca fluviatilis*) were exposed to ship noise. The noise level was well above hearing thresholds in these species. In a second series, fish were exposed to continuous Gaussian noise at a similar level (156 dB) which is known to induce temporary hearing loss in hearing specialists. **All three species responded with increased cortisol secretion when exposed to ship noise. Interestingly, no elevation was observed when fish were exposed to continuous Gaussian noise.** Our results indicate that ship noise characterized by amplitude and frequency fluctuations, **constitutes a potential stressor** in contrast to continuous noise. **Surprisingly, the data also demonstrate no apparent differences between species possessing excellent hearing abilities (hearing specialists) and species with poor hearing abilities like perch.**

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## 1. Introduction

Although extensive research has been done on various stressors in fish like aquaculture, fishery, tourism, and social factors (e.g., Fox et al., 1997; Iwama et al., 1997; Haddy and Pankhurst, 1999; Pottinger et al., 1999; Øverli et al., 1999; Oliveira et al., 1999; Sakakuro and Tsukamoto, 1999), there has been little focus on the effects of noise. The latter is particularly important because anthropogenic activities such as commercial shipping, recreational activities, drilling, seismic exploration or

energy production (hydroelectric power plants), have made underwater noise pollution an increasing and perhaps dominant factor in the aquatic environment (Myrberg, 1990; Andrew et al., 2002; Popper, 2003; Amoser et al., 2004).

Anthropogenic noise can alter the behavior of whales, birds and fish and thus have long term direct or indirect consequences on the behavior, fitness and ecology of a species. Certain whale species react to approaching vessels by changing their vocalizing, resting and migration behavior (Richardson et al., 1995; Lesage et al., 1999). Recreational activities, like

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boat fishing, have been found to be a major disturbance in several species of migrating water birds resulting in increased alertness, escape activities and energy expenditure (Schummer and Eddleman, 2003). The effects of boat noise on fishes have mainly been investigated in the marine environment within the framework of population assessment and better management of catch rates for the fishing industry. **Avoidance reactions to vessel noise of marine species such as herring (*Clupea harengus*) and cod (*Gadus morhua*) and the freshwater fishes rudd (*Scardinius erythrophthalmus*) and roach (*Rutilus rutilus*) have been studied in the field (Boussard, 1981; Świerzowski, 1999; Vabø et al., 2002; Mitson and Knudsen, 2003; Handegard et al., 2003). It was shown that fishes actively avoid specific kinds of vessels with vertical and horizontal displacements.**

There is some indication that noise can elicit an endocrinological stress response in fish as well. Sverdrup et al. (1994), Santulli et al. (1999) and Smith et al. (2004) reported changes in cortisol and other biochemical parameters in Atlantic salmon (*Salmo salar*) and European sea bass (*Dicentrarchus labrax*) after air-gun detonation or underwater explosions and in goldfish (*Carassius auratus*) after exposure to 160 dB white noise.

The aim of the present study was to investigate whether and to what degree ship noise encountered in rivers and lakes could produce a similar cortisol increase in three selected species of common European freshwater fishes. Cortisol is considered as the principal corticosteroid secreted by the teleost fish adrenal system in response to acute and chronic stress (Mommensen et al., 1999). Its concentrations in the blood, plasma, tissue, and recently also in the holding water has been commonly used to monitor stress responses (e.g., Jeney et al., 1992; Pickering, 1992; Gamperl et al., 1994; Pottinger et al., 1999; Scott et al., 2001; Ellis et al., 2004).

We exposed fish to ship noise recorded in the field and to continuous Gaussian noise with a relatively equal energy distribution over a broad frequency spectrum. Both noise types were then applied at physiologically relevant levels known to result in temporary hearing loss in fish species with specialized hearing (Amoser and Ladich, 2003; Smith et al., 2004; Wysocki and Ladich, 2005). In the present experiment, we chose species with quite different hearing abilities to test whether hearing specialist species like common carp (*Cyprinus carpio*), and gudgeon (*Gobio gobio*) with excellent hearing abilities were more affected by noise than a hearing generalist, the European perch (*Perca fluviatilis*). Similarly, we hypothesized that a dynamic and thus unpredictable noise source with varying levels and frequency content like that associated with the passage of various vessels in the field would elicit a different and perhaps more intensive stress response than more consistent noise to which an organism could possibly become habituated.

## 2. Methods

### 2.1. Animals

Test subjects were seven European perches (*P. fluviatilis*) (79–98 mm standard length (SL); 8.3–14.9 g body mass (BM)) obtained from a hatchery at Lake Mondsee (Scharfling, Upper

Austria), six common carps (*C. carpio*) (99–138 mm SL, 32.1–68.9 g BM) from a fish pond near Vienna, and seven gudgeons (*G. gobio*) (76–101 mm SL; 6.9–13.3 g BM) from local pet suppliers.

All animals were kept in planted aquaria with sand-covered bottoms and halved flower pots as hiding places. Water was filtered by external filters, and light maintained in a 12L:12D cycle. The fish were fed live *Tubifex* sp., chironomid larvae or commercially prepared flake food (Tetramin, Tetrapond®, Dana Feed®) daily. All experiments were performed with the permission of the Austrian Commission on Experiments in Animals (GZ 68.210/50-Pr/4/2002).

### 2.2. Experimental setup

Each individual was subjected to three different test conditions: Boat noise, Gaussian noise, and no-noise control. In each test condition, a single fish was placed in a plastic bucket (21 cm height, 22 cm diameter, 12 cm water depth) containing 3 l of water for 30 min. The water in the bucket originated from a 200-l storage water tank, that did not contain any fish. Cortisol levels were measured in 1 l samples of the storage tank water to control for the potential presence of waterborne substances that could bind to the cortisol antisera used. The levels of immuno-reactive cortisol in these samples were below the detection limit of the assay. The mean temperature during experiments was  $22 \pm 1$  °C.

After the noise exposure period, the fish were removed from the bucket and 1 l of water was taken and subjected to the cortisol extraction procedure described below. Animals were not fed for at least 20 h prior to experiments. In order to avoid any additional manipulations (and potential influences on the endocrinological response) on the fish during the experiments such as the presence of an observer and/or handling of a video system, we did not perform simultaneous behavioral observations of the fish. An additional reason was that in this restricted environment, the fish would not have been able to display a behavioral repertoire similar to that expected in the field.

Two sets of experiments were done per day using different individuals. To control for diurnal hormone fluctuations (Oliveira et al., 2001), the first set of experiments took place between 9:30 and 10:00 a.m. (one and a half hour after light onset) and the second shortly afterwards between 11:45 a.m. and 12:15 p.m. Efforts were taken to re-measure the same individuals at the same time of day, thus 70% of all individuals were compared at exactly the same time of day while in the remaining cases, no-noise control tests either took place slightly earlier or later than noise exposure tests. For the no-noise measurements, the influence of experimental time was examined but no effect was found in any of the species tested (Mann–Whitney *U*-tests, European perch:  $Z = -0.867$ ,  $p = 0.386$ ,  $n = 14$ ; common carp:  $Z = -0.809$ ,  $p = 0.418$ ,  $n = 12$ ; gudgeon:  $Z = -0.518$ ,  $p = 0.605$ ,  $n = 14$ ).

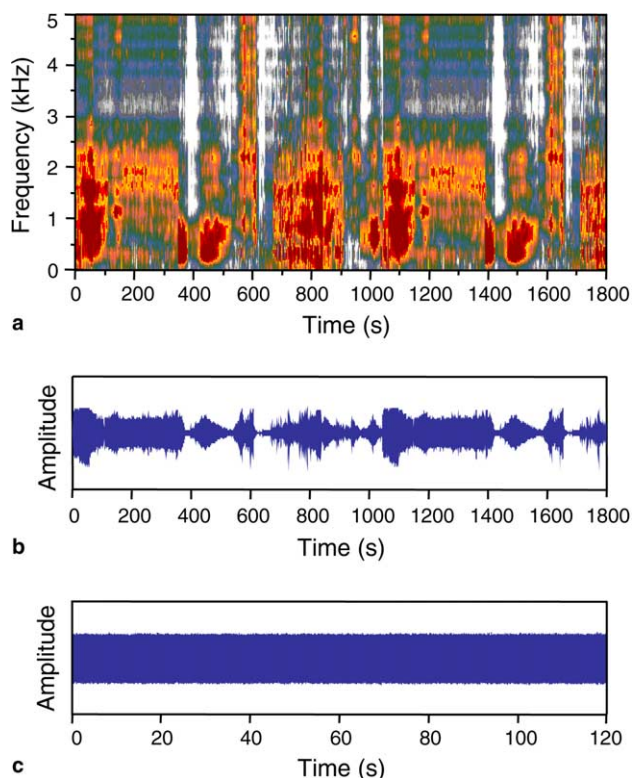
The equivalent continuous sound pressure level ( $L_{Leq}$ ) in the bucket, averaged over 30 min, was measured using a sound level meter (Brüel & Kjær 2238 Mediator), a power supply (Brüel & Kjær 2804) and a hydrophone (Brüel & Kjær 8101). The  $L_{Leq}$  is a measure of the averaged energy in a varying sound level and is commonly used to assess environmental noise (ISO 1996).

During the month preceding the experiments, fish were familiarized with the test situation by placing them for 30 min in the experimental bucket. This was done to alleviate the potential stress of environmental changes.

### 2.2.1. Treatment 1: Ship noise exposure

The noise emitted during the passage of boats was recorded on different days and sites in the Danube River and the lakes Mondsee and Traunsee, using a hydrophone (Brüel & Kjær 8101) connected to a power supply (Brüel & Kjær 2804) and a DAT recorder (Sony TCD 100). A 17-min WAV-file (Fig. 1(a) and (b)) was created in Cool Edit (version 2000) and played back to the fish for 30 min in a loop. The playback noise was sent through a 30-band equalizer (Alesis MEQ 230), adjusted so that the spectral characteristics of the boat noise matched the original recording, and fed to a power amplifier (Brüel & Kjær 2713) that drove an underwater loudspeaker (University Sound UW 30) situated on the bottom of the bucket. The  $L_{Leq}$ , averaged over the 30-min period was 153 dB (instantaneous levels ranging from 128 to 162 dB).

Control measurements (boat noise controls) were performed prior (mean interval  $9 \pm 1$  days) to boat noise exposures in order to determine “baseline” cortisol values in the test bucket. For controls, the same individuals were placed for 30 min in the plastic bucket without turning the noise



**Fig. 1** – (a) Sonagram (filter bandwidth 100 Hz, 50% overlap, sampling frequency 44.1 Hz, window: Blackman–Harris) and (b) oscillogram of the boat noise played back to the fish as recorded in the test bucket. (c) Oscillogram of a segment of the Gaussian noise played back to the fish as recorded in the test bucket. Compare waveforms in (b) and (c) in order to see amplitude fluctuations.

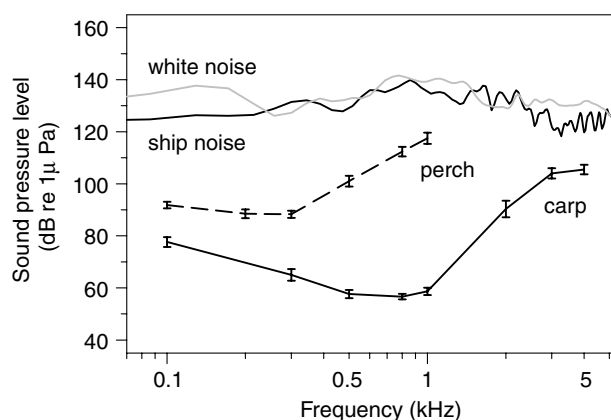
on. Experiments were performed from the 10th of March 2004 to the 15th of April 2004 for carp and perch, and from the 5th of May 2004 to the 25th of May 2004 for gudgeons. A large time span between control and experimental measurements was chosen to ensure the independence of each data set.

### 2.2.2. Treatment 2: Gaussian noise exposure

Gaussian (White) noise was generated by a noise generator (IVIE Electronics IE 20B), sent through a 30-band equalizer (Alesis MEQ 230) to obtain a relatively flat spectrum (Fig. 2) up to the upper hearing limit of the hearing specialists (5 kHz) and played back to the fish for 30 min. The equivalent continuous SPL ( $L_{Leq}$ ), averaged over 30 min was 156 dB. White noise controls were measured  $7 \pm 1$  days prior to noise exposure experiments. Experiments were performed from the 5th of August 2004 to the 13th of September 2004. The rationale for the time interval between tests was as mentioned above.

### 2.3. Hormone extraction and data analysis

The cortisol extraction procedure of the water was modified after Scott and Sorensen (1994), Scott et al. (2001), Oliveira et al. (2003) and Hirschenhauser et al. (2004). To exclude contamination, all materials and sample containers used were washed with distilled water before and after sampling. One-liter samples of water were decanted from the test bucket into silanized glass beakers and then filtered through paper filters (24 cm diameter) to remove particulate matter. They were then drawn by means of a vacuum pump through solid phase extraction columns (LiChrolut<sup>®</sup> RP-18, [40–63  $\mu$ m; 500 mg of 3 ml standard PP-tubes], Merck KgaA, Darmstadt) which had been previously “activated” with 4 ml of 96% ethanol followed by 4 ml of distilled water. The flow rate was approximately 1 l/h. After the extraction, steroids were eluted from the cartridges with 4 ml of 96% ethanol. The ethanol was evaporated over night in vacuum at 34 °C. The residue



**Fig. 2** – Audiograms (mean  $\pm$  SE) of common carp (solid lower line) and European perch (dashed lower line) (data from Amoser et al., 2004) and averaged frequency spectra of the ship noise (solid upper line) and Gaussian noise (gray upper line) played back in the laboratory.

(extracts) were re-dissolved in 1 ml phosphate buffer and stored in a freezer at  $-18^{\circ}\text{C}$ .

The excretion rates of fish are expected to be related to body mass and the fact that the ratio of gill surface area to body mass decreases with increasing size (Pauley, 1994). For this reason smaller fish would potentially release more cortisol with a similar plasma cortisol concentration as larger fish. The gills appear to be a major source of steroids released into the water (Vermeirssen and Scott, 1996; Scott et al., 2001). Therefore, the measured cortisol levels were corrected for body mass and expressed as ng/l water/g fish.

Cortisol levels were determined in ng cortisol/l water/g fish by enzyme immunoassay (EIA) with DADDO (diamino-dioxo-octane)-biotinylated cortisol-3-CMO labels and bovine serum albumin-coupled antibodies (dilution 1:20) against cortisol-3-CMO (carboxy-methyloxime); the characteristics of the EIA procedures applied and the cross-reactions of the antibodies used have been described elsewhere (Palme and Möstl, 1997). Standard pools of high and low cortisol were put into each assay to determine interassay variation and validate interassay comparisons. The lowest detectable limit of the assay was 0.13 pg/assay unit. The intra-assay coefficients of variation based on the duplicate sample measurements were 15.6% for European perch, 10.5% for common carp, and 12.3% for gudgeon. No inter-assay coefficients of variation were determined because each species was analyzed in a separate individual EIA run.

Due to the fact that data were not normally distributed and the small sample size non-parametric tests were used. Cortisol levels between no-noise controls and the noise-exposure situations were compared within each individual using Wilcoxon matched-pairs tests. All statistical tests were run using SPSS version 12.0.

### 3. Results

It was possible to document cortisol in the tank water inhabited by all the species studied. As described below these levels were always above 0.1 ng/l water/g fish. The levels of cortisol in storage water were below the level of detectability in our assay, hence they were less than 2.6 pg/l water. Hence the presence of fish was associated with at least a 280-fold increase in cortisol concentration for a 7-g fish. In the experiment cortisol levels following a 30-min exposure to boat

noise were significantly higher (Fig. 3(a)) than control levels in all three species (Wilcoxon matched-pairs tests: European perch:  $Z = -2.366$ ,  $p = 0.018$ ,  $n = 7$ ; common carp:  $Z = -2.201$ ,  $p = 0.028$ ,  $n = 6$ ; gudgeon:  $Z = -2.366$ ,  $p = 0.018$ ,  $n = 7$ ). The mean increase in cortisol was about 99% over control values in European perch, 81% in common carp, and 120% in gudgeon. Exposure to Gaussian noise did not produce a significant change in cortisol levels relative to the controls (Fig. 3(b)) (Wilcoxon matched-pairs tests, n.s. for all species investigated).

The no-noise control levels were similar in both control measurements for common carp (average of  $0.22 \pm 0.05$  ng/l water/g fish for boat noise controls versus  $0.16 \pm 0.01$  ng/l/g for Gaussian noise controls) and the European perch ( $0.15 \pm 0.01$  ng/l water/g fish boat noise,  $0.25 \pm 0.07$  ng/l water/g fish Gaussian noise). The control levels in gudgeon differed being  $0.34 \pm 0.09$  ng/l water/g fish for boat noise but  $0.1 \pm 0.04$  ng/l water/g fish for Gaussian noise controls. Although the cortisol measurements for each individual species were done in separate runs of the assay, it is reasonable to assume that these differences in control results represent species differences in basal cortisol secretion patterns.

## 4. Discussion

### 4.1. Hormone determination in the aquarium water

Assessing the stress response in any animal is always difficult since the investigation procedures themselves can induce stress. This is especially the case when manipulation of the subjects is required, i.e., by catching and bleeding them in order to assess biochemical parameters in the blood. Procedure-induced stress has the potential to mask the effects of the stressors in question. Therefore, non-invasive methods such as extracting hormones from feces samples or – in the case of aquatic animals – the holding water, have been suggested as invaluable alternatives to the classic, more invasive methods for the measurements of steroid hormones (Scott and Sorensen, 1994; Oliveira et al., 1999, 2003; Scott et al., 2001; Hirschenhauser et al., 2002, 2004; Turner et al., 2003; Ellis et al., 2004). In addition, non-invasive procedures can help to minimize the effect of individual differences in cortisol concentrations related to the so-called coping styles (Laidley and Leatherland, 1988; Lepage et al., 2000) by permitting

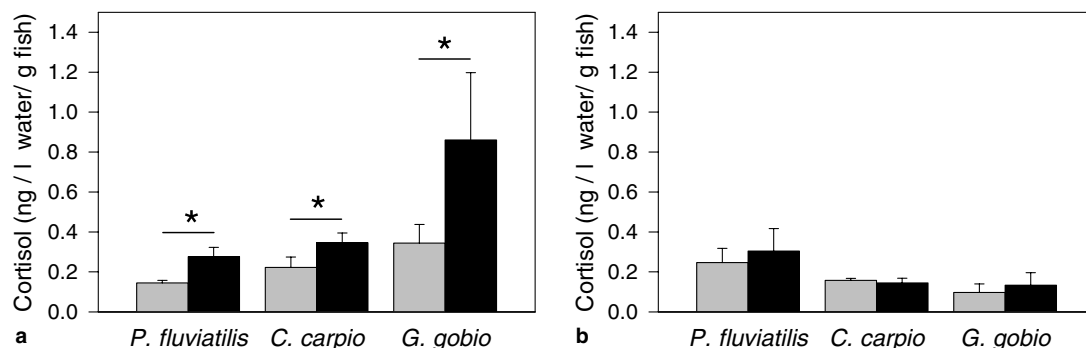


Fig. 3 – Mean ( $\pm$ SE) cortisol levels of the three species investigated when exposed to (a) ship noise (black bars) and no-noise control (gray bars) and to (b) Gaussian noise (black bars) and no-noise controls (gray bars).

repeated tests on particular individuals in different experimental conditions.

Scott and Sorensen (1994) and Moriwaki et al. (1991) have compared water and plasma steroid concentration in HCG-injected female goldfish. Their results demonstrated a good match in the rise and fall of 17,20 $\beta$ -P and testosterone. Similarly, Hirschenhauser et al. (2004), injected male tilapia with LHRH and showed that androgen measurements in fish-holding water reliably reflected systemic levels. An earlier study subjecting rainbow trout (*Oncorhynchus mykiss*) to single or multiple stressors (Scott et al., 2001) also had demonstrated that cortisol is released into water by fish after stressor application in amounts sufficient for detecting differences associated with the treatment. Ruane and Komen (2003) measured cortisol levels in the plasma and water of common carp subjected to different loading densities used in commercial aquaculture. In both cases, cortisol levels increased with loading densities. The increases in cortisol after loading were present for even a longer period of time in the water than in the plasma. In summary, a number of experiments have demonstrated that measuring cortisol levels in ambient water is useful in estimating the stress responses in fish. In one report, however, Ellis et al. (2004) recently showed that several variables such as the loss of cortisol in static water compared to running water or the elution method used might affect absolute cortisol levels measured and bias the results. While these and other factors affecting release may potentially reduce the applicability of this method as an absolute measure of the degree of stress in individuals, it is nonetheless useful as a relative measure to monitor the immediate and individual effects of a potential stressor.

In the present study the technique was used to test for effects of different types of noise on the secretion of cortisol. The goals were not to document absolute levels of adrenal activity or to elucidate the neuro-biochemical mechanisms underlying the response. Our data show that by the method applied, cortisol can be extracted from the holding water in amounts sufficient to reflect variations in secretion related to particular test situations and the species investigated. Since other exogenous factors such as light–dark regime, feeding time, and water temperature were kept as constant as possible, they only played a minor (if any) role in glucocorticoid secretion and metabolism in water. Therefore, the variations observed in cortisol levels reflected a relatively acute response to the stressor (exposure to boat noise) compared to unstressed/control conditions.

Although baseline data are not easily compared among species, the results do show clear inter-species differences. While the control values of common carp remained most stable during both test series, they increased in European perch and decreased in gudgeon. These control variations may be due to seasonal variations in hormone levels known to occur in various fish species (e.g., Rinchard et al., 1993) since the second test series was performed 4–5 months after the first.

#### 4.2. Noise as a potential stressor

Noise of anthropogenic origin constitutes an increasing environmental problem not just in the terrestrial but also in aquatic environments. While much concern has been dedicated to

the impacts of human activities emitting noise on physiology, behavior, acoustic communication and reproductive success of terrestrial animals (e.g., Reijnen et al., 1996; Gibeau et al., 2002; Dyck and Baydack, 2004; Sun and Narins, 2005) and marine mammals (Richardson et al., 1985, 1995; Myrberg, 1990; Lesage et al., 1999; Nowacek et al., 2004), public interest has begun to focus on fish and freshwater communities, too (Scholik and Yan, 2002; Popper, 2003; Popper et al., 2004, 2005; Amoser et al., 2004).

Noise can mask an animal's hearing or induce temporary hearing loss and thus impair the detection of predators and prey, acoustic communication, and orientation. Noise can also elicit a physiological stress response in numerous animals. This response has been shown to range from changes in pulse rate (Waynert et al., 1999) and effects on growth and development (Myrberg, 1990; von Holst, 1998) to alterations of basic physiological processes in organisms like increases in cortisol or changes in thyroid function (e.g., van Raaij et al., 1996). The effects of noise on biochemical stress responses have, to date, mainly been investigated in mammals, a considerable amount of which has dealt with aspects related to human health (for a review see Spreng, 2000).

Far fewer data are available on other vertebrate groups. Two studies have investigated endocrinological stress responses in fish (Sverdrup et al., 1994; Santulli et al., 1999) to high-intensity seismic exploration sounds. Shipping noise is less intensive but much more abundant than explosions in both the marine and freshwater environments. Our data confirm that noise can produce increased cortisol secretion in three common freshwater species. In contrast to expectation, this increase was independent of the individual species' hearing abilities. European perch showed a stress response similar to otophysines despite their restricted hearing sensitivity (Fay, 1988; Amoser et al., 2004; see also Fig. 2). On the other hand, continuous Gaussian noise of comparable intensity did not increase cortisol levels in any of the species even though the sound level was high enough to induce temporary hearing loss in the otophysines (Scholik and Yan, 2002; Amoser and Ladich, 2003). In our experiments the equivalent continuous SPL at even higher levels (156 dB vs. 153 dB) did not produce a significant response. It seems therefore, that a less predictable stimulus, fluctuating in both amplitude and frequency, is more likely to activate the HPA-axis than a constant stimulus. The ability to adapt to continuous noise is important since many fish species, e.g., gobies live and reproduce in inherently noisy natural habitats such as rocky creeks, torrents and seashores (Lugli and Fine, 2003) and must therefore be able to maintain their normal activities despite high levels of background noise.

A final factor to consider is the time course of hormone release. A number of studies on stress responses in fish have shown that reactions occur very quickly, i.e., significantly elevated plasma cortisol can be found 5 min after an acute stressor (a fight) (Øverli et al., 2004). Ellis et al. (2004) demonstrated a 7-fold increase in water cortisol concentration within 30 min of stressor application, indicating that the time lag between plasma and water cortisol is only a matter of minutes although the peak of cortisol secretion was found one hour after the stressor. We were able to detect significantly elevated cortisol levels in the water 30 min after the stressor

onset. It is possible that the levels would have risen further over a longer time period if investigated. Our main aim was, however, to detect whether short-time boat noise, which is frequently encountered in the field had the potential of being a stressor for fish.

#### 4.3. Conclusions and implications for conservation

It is often empirically difficult to causally link human activities to specific changes in animal behavior. To date, the impacts of navigation have mainly been linked to hydraulic forces challenging swimming performance in juvenile freshwater fish, or indirect effects imposed by the development of suitable waterways such as migration barriers, pollution, habitat loss, and biotope simplification (Wolter and Arlinghaus, 2003). The factor noise and its impact on fish behavior and physiology has been neglected with only a few exceptions. Active avoidance of vessels in dependence of the amount of noise emitted have been found in cod, herring, and also in the freshwater species rudd and roach (Boussard, 1981; Vabø et al., 2002; Mitson and Knudsen, 2003; Handegard et al., 2003). These individual observations only allow to speculate on the impacts of noise on the population. Boussard (1981) observed that fish stopped spawning activity when high-speed boats were approaching. The field observations and subsequent studies in the lab confirmed that this reaction was solely due to the noise emission of the boats.

In summary, our results show that ship noise can elicit a cortisol stress response in different species regardless of their hearing sensitivities. This is relevant because underwater noise pollution is a growing environmental problem and some evidence exists that noise disrupts developmental processes and growth in aquatic organisms including fish (Banner and Hyatt, 1973; Myrberg, 1990). Cortisol has detrimental effects on growth, sexual maturation and reproduction, immunological function and survival in fish (Pickering, 1992; McCormick, 1999; Pankhurst and Van der Kraak, 2000; Consten et al., 2001a,b, 2002; Ellis et al., 2002). Stress challenges an organism's homeostasis acting thus as a threat to its health (Barton and Iwama, 1991) and the level of stress can serve as an important welfare indicator.

Based on our data and on the fact that intermittent ship noise usually goes on for a longer time than the period used in our experiments, more future long-term studies should be directed towards a multifactorial analysis of ship trafficking effects on fish. It is necessary to expand these studies to include analyses of fish behavioral responses to different types of vessels in the field. In addition, hydrology, season, community composition, and habitat structure should be considered as compounding factors that could affect fish populations. These studies could then be used to develop plans for effective protection. Onboard noise levels have become a significant element in the construction and design of ships in recent years (Vasconcellos and Latorre, 2001) with regard to passenger comfort and navigation safety. We would suggest that similar effort should be taken to lessen the underwater noise emission. Ships in freshwater should be required to reduce their noise levels with similar techniques as had been prescribed in the ICES CRR 209 recommendations for research vessels in marine environments (Mitson, 1995).

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