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Aspects of lentic fish behaviour studied with high resolution positional telemetry

Ph.D. dissertation

Henrik Baktoft

May 2012

Technical University of Denmark

National Institute of Aquatic Resources

Section for Freshwater Fisheries and Ecology

Preface

This dissertation is submitted in partial fulfilment of the requirements for obtaining the degree of Doctor of Philosophy (Ph.D.) at the Technical University of Denmark (DTU). The thesis represents work conducted during a three year scholarship in the period 2009 – 2011 at DTU, National Institute for Aquatic Resources (DTU Aqua), Section for Freshwater Fisheries and Ecology. The project was funded by the Danish Rod and Net Fishing License Funds and DTU.

As indicated by the broad title, the present thesis does not intent to answer a single specific question. Fish behaviour can be surprisingly complex and is influenced by numerous exo- and endogenous factors. Consequently, the study of fish behaviour and what affects it can require the integration of several biological disciplines, such as methods to quantify behaviour in the field and laboratory as well as physiological measures on metabolic properties. I chose to work with a combination of these approaches for two main reasons: 1) to gain and increase experience with various biological tools and to obtain a better overview of the integrative field of fish behaviour. 2) An inter-disciplinary approach was required to go beyond solely descriptive studies in attempts to establish potentially explanatory relationships.

During my work, I have been surrounded by a great number of helpful people to whom I am indebted. Firstly, I would like to thank Lene Jacobsen (main supervisor) for all her support, help and continued confidence in the project, regardless of the crazy ideas I have suggested. I am equally grateful to the team of co-supervisors (Kim Aarestrup, Søren Berg, Anders Koed, Jon C. Svendsen and Christian Skov) for always being available for discussions, suggestions, quick advices, incentive talks and early stopping of occasional wild goose chases. Additionally, Niels Jepsen has, in spite of being the only senior scientist in the section not enrolled as a supervisor, helped as if he was.

In the course of this work, I had the privilege to visit professor Robert Arlinghaus and his group at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany. They are all thanked for an inspirational stay including accommodation, scientific discussions and collaboration, field work and great German beer.

Everybody at DTU Aqua, Silkeborg is thanked for support and for maintaining an enthusiastic and informal atmosphere. In particular, the technical personnel have provided excellent help when needed; among countless things, Morten Carøe and Jørgen Skole Mikkelsen helped with field work and tagging of fish; Birgit Therkildsen assisted with aging of fish through scale reading; Michael Holm took the chore of regular sampling and monitoring of the study site; Jes Dolby made it possible to retrieve data from Lake Gosmer from the comfort of the office; finally, Hans Jørn Christensen utilized his exceptional angling skills and provided good company when sampling for pike, often during unsociable hours.

I am very grateful for the Hidden Markov Model provided by Martin Wæver Pedersen (daily referred to as “the Wæver Filter”), which enabled an objective and standardised way to make sense of the raw data from the telemetry system.

The owner of Lake Gosmer, Søren Anton Hansen and family, are deeply thanked for allowing the project while maintaining and enforcing a ‘no access policy’ to the lake.

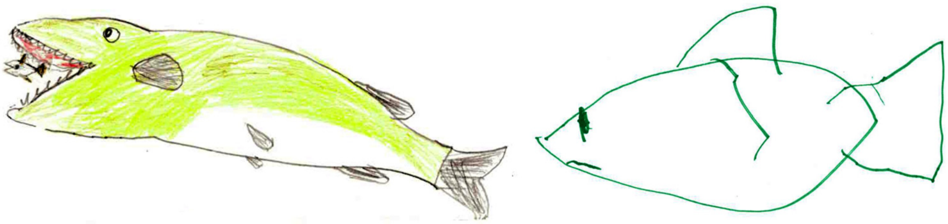
I thank Lotek Wireless Inc. for teaching me invaluable lessons in the important personal skill of patience by providing transmitters for a particular study that rendered several months of lab work futile due to an extreme transmitter failure rate (> 80 %).

Special thanks are owed to fellow Ph.D. and master students at DTU Aqua, Silkeborg for friendship and fun. Especially to: Mikkel Boel for discussions and companionship on several statistical courses; Kristian Meier for occasional, but way too few, fishing trips and Diego Del Villar for making the Japan trip memorable.

Lastly, I will take the opportunity to thank my family and friends for moral support and for picking up the kids from kindergarten/school when needed. Above all, I am very grateful for love and support from my wife, Karen, and our two kids, Anna and Aske; for giving me the time needed when this project required my full attention and for reminding me that there is more to life than fish behaviour and telemetry.

Henrik Baktoft

Silkeborg, May 2012



Fish behaviour and morphology (courtesy of Anna and Aske Baktoft)

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List of original manuscripts

I **Baktoft, H.**, Zajicek, P., Klefoth, T., Svendsen, J.C., Jacobsen, L., Pedersen, M.W. & Arlinghaus, R. (*In prep*). Assessing the accuracy, precision and efficiency of two automated acoustic telemetry systems (APTS) covering two freshwater lakes.

II **Baktoft, H.**, Aarestrup, K., Berg, S., Boel, M., Jacobsen, L., Jepsen, N., Koed, A., Svendsen, J.C. & Skov, C. (2012). Seasonal and diel effects on the activity of northern pike studied by high resolution positional telemetry. *Ecology of Freshwater Fish.* Online version available.

III Jacobsen, L., **Baktoft, H.**, Jepsen, N., Aarestrup, K., Berg, S. & Skov, C. (*In prep*). Effect of anthropogenic disturbances on lake fish individual behaviour.

IV **Baktoft, H.**, Aarestrup, K., Berg, S., Boel, M., Jacobsen, L., Koed, A., Pedersen, M.W., Svendsen, J.C. & Skov, C. (*Submitted*). Effects of angling and manual handling on pike behaviour investigated by high resolution positional telemetry. *Fisheries Management and Ecology. Special issue: Proceedings of the Eight Conference on Fish Telemetry held in Europe, Umeaa, Sweden 2009*.

V **Baktoft, H.**; Aarestrup, K.; Boel, M.; Jacobsen, L.; Skov, C.; Svendsen, J.C. (*In prep*). Can metabolic or morphologic properties explain variation in individual behaviour? Linking physiology and morphology with field behaviour.

Co-authored papers not included in the thesis

In the course of the present work, I have been involved in several other research projects, part of which has been published. These papers are referred to where appropriate but are not to be evaluated for submission of this thesis.

Nilsson, P. A., **Baktoft, H.**, Boel, M., Meier, K., Jacobsen, L., Rokkjaer, E. M., Clausen, T., & Skov, C. (2012). Visibility conditions and diel period affect small-scale spatio-temporal behaviour of pike *Esox lucius* in the absence of prey and conspecifics. *Journal of Fish Biology* 80, 2384-2389.

Skov, C., Aarestrup, K., **Baktoft, H.**, Brodersen, J., Brönmark, C., Hansson, L. A., Nielsen, E. E., Nielsen, T., & Nilsson, P. A. (2010). Influences of environmental cues, migration history, and habitat familiarity on partial migration. *Behavioral Ecology* 21, 1140-1146.

Skov, C., **Baktoft, H.**, Brodersen, J., Brönmark, C., Chapman, B., Hansson, L. A., & Nilsson, P. A. (2011). Sizing up your enemy: individual predation vulnerability predicts migratory probability. *Proceedings of the Royal Society B-Biological Sciences* 278, 1414-1418.

Svendsen, J. C., Aarestrup, K., Malte, H., Thygesen, U. H., **Baktoft, H.**, Koed, A., Deacon, M. G., Cubitt, K. F., & McKinley, R. S. (2011). Linking individual behaviour and migration success in *Salmo salar* smolts approaching a water withdrawal site: implications for management. *Aquatic Living Resources* 24, 201-209.

Introduction

Why study fish behaviour?

Scientists have been studying animal behaviour for decades for several reasons. Firstly, animal behaviour is intrinsically interesting to observe. Secondly, observational studies on animal behaviour very frequently spawn a desire to do other studies, attempting to answer the intriguing question "why do these animals behave as they do?" Additionally, from a management point of view it can be argued that knowledge about animal behaviour is essential in order to e.g. enable a sustainable exploitation of the resource constituted by the animals or in order to avoid extinction of species endangered by human activities. This is true for species in most, if not all, taxa in the animal kingdom; fish being no exception.

> *"Gone, or, at least, redundant, are the days where fishes were looked down upon as pea-brained machines whose only behavioural flexibility was severely curtailed by their infamous three-second memory".* (Brown et al. 2006)

What influence fish behaviour?

The behaviour of a fish in a given situation can be viewed as a complex response to the interplay of a wide array of external and internal stimuli and factors (Fig. 1). Some of the external factors can have a direct and immediate effect (e.g. predation and angling) whereas others have more subtle and indirect effects (e.g. a behavioural response to changes in water temperature). Additionally, intrinsic factors such as morphology and physiology can affect behaviour on an individual level. Obviously, a thorough and detailed description of these influential factors and their potential interplay is beyond the scope of this work. Instead, I have focused on a few central topics attempting to answer the question "how does this particular factor affect the behaviour of fish?" To study this question, high resolution telemetry was used to 'observe' and record the behaviour of individually tagged fish.

About this dissertation

This dissertation presents work carried out in 2009 – 2012. The majority of the presented data originate from an advanced telemetry system deployed in a small lake inhabited by northern pike (*Esox lucius*), roach (*Rutillus rutillus*), European perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*). These species are henceforth referred to as pike, perch, roach and eel. The system enabled research on the detailed volitional behaviour of tagged fish in their natural environment. In short, the system provided time stamped two or three dimensional positions (dependent of transmitters used) of tagged fish with high spatial and temporal resolution. By tagging the majority of larger pike (> 35 cm) in the study lake, as well as groups of roach and perch, we obtained unique time series on their behaviour. For instance, at the time of writing, several of the tagged pike have been continuously monitored for a period exceeding three years.

The dissertation structure follows Fig. 1 with focus on highlighted topics. First section presents the applied telemetry system and main results from a study evaluating the performance of the system. The following sections elaborate on three factors affecting fish behaviour (environmental factors, human activities and individual variability) through a mixture of general introduction to each subject and new insights gained from this work. Most of the results presented can be found in the included manuscripts, referred to by their roman number enclosed in slashes (e.g. ms/IV/). Additionally, some results not (yet) materialised in manuscripts are included in this thesis for completion.

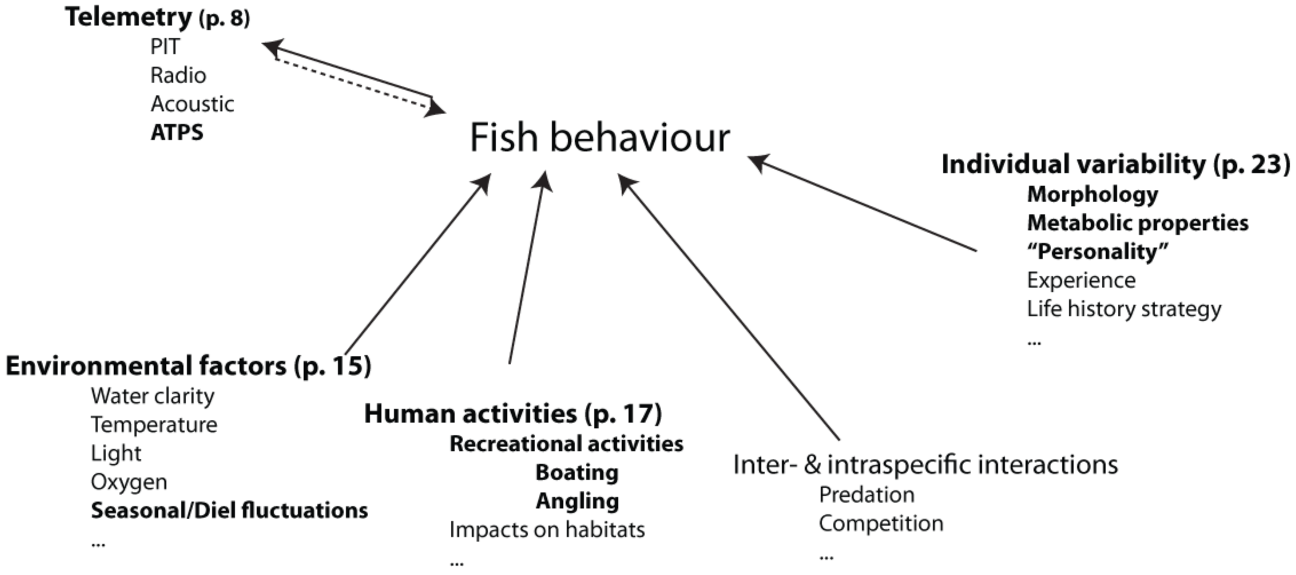


Fig. 1. Thesis structure depicted as a conceptual model of factors affecting fish behaviour. Factors discussed in the present work are highlighted in bold.

Methodology

Acoustic Positional Telemetry System (APTS)

The majority of work in the present thesis is based on the utilization of an acoustic positional telemetry system (APTS) deployed in a small lake (Lake Gosmer, Denmark, 55°55´42 N, 10°10´50 E, Fig. 2, area 1 ha, max depth 8 m, mean annual secchi depth 1.2 m, range 0.4 m – 2.4 m) to study the behaviour of fish in natural settings.

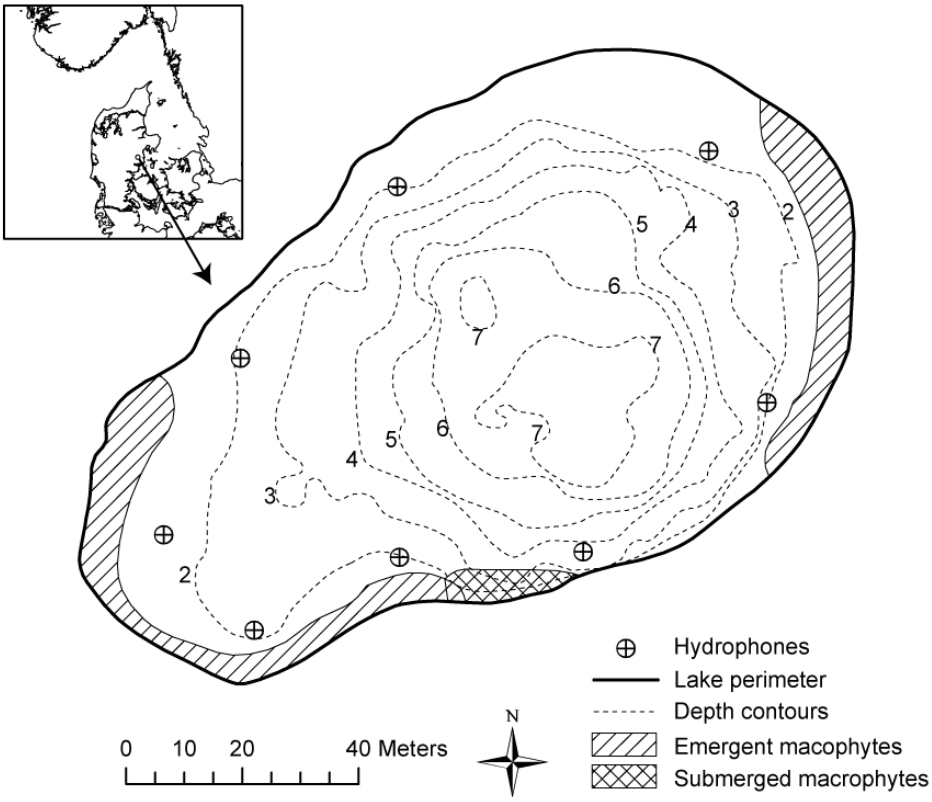


Fig. 2. Outline of Lake Gosmer.

Telemetry technologies are widely used to acquire information on individual fish behaviour that would otherwise be unobtainable due to the challenges inherent in the study of underwater animals (Lucas & Baras 2000; Block 2005). Whereas traditional telemetry technologies, such as radio and acoustic telemetry, have an inherent trade-off between spatial and temporal resolution, APTSs potentially offer the best of both worlds, i.e. both high spatial and high temporal resolution. Furthermore, when using an APTS, tagged fish are monitored automatic by data loggers. Thus, the potential disturbance of the focal fish caused by the manual tracking protocol typically used in traditional telemetry methods is avoided. The APTS rationale is based on arrays of multiple synchronized hydrophones located at known positions. These hydrophones survey the study area, detect acoustic signals emitted from deployed transmitters and store the information. The temporal resolution of the data is thus determined by the burst interval of the used transmitters, typically sub-minute; transmitters with 2.5, 5, 15, 30 and 45 second burst intervals were used during this work. Subsequently, acquired data are post processed to obtain time stamped geographic positions using hyperbolic triangulation. Given that an acoustic signal from a transmitter is detected by at least three hydrophones, the position of the transmitter at the time of signal emission can be calculated from the minute differences in time of arrival at each hydrophone (Box 1). The achievable spatial resolution is a product of multiple complex parameters such as the geometric layout of the hydrophone array and the position of the transmitter relative to the hydrophones detecting the signal. Under optimal conditions sub-meter accuracy is achievable (Niezgoda et al. 2002, but see below and ms/I/).

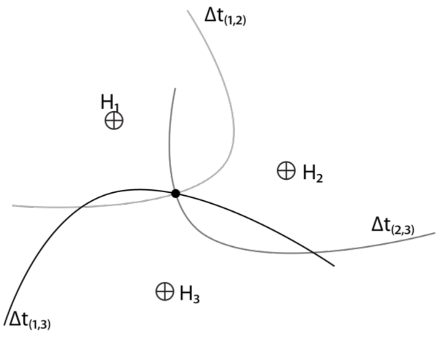


Figure (not exact) illustrating the TDOA principles. Transmitter position is indicated by the black dot, hydrophones by circled crosses (H_1, H_2 and H_3). Hyperbolic curves (e.g. $\Delta t_{(1,3)}$) indicate constant TDOA trajectories for each pair of hydrophones. (Redrawn from “BioMap Positioning for MAP”; Lotek Wireless 2005)

BOX 1

Positioning based on “time differences of arrival” (TDOA)

Time of arrival positioning is based on the fundamental relationship between velocity, distance and time. As acoustic waves travel at a relatively constant velocity in water ($c \approx 1500$ m/sec) the distance between a hydrophone and transmitter can be calculated from the time difference between signal emission and detection. However, this rationale is only applicable if the transmitter and hydrophones are perfectly synchronized and the exact time of signal emission is known. As this is virtually impossible to achieve in field situations, the rationale is extended to facilitate calculation of the transmitter position without knowing the exact time of signal emission. By comparing the arrival times of the same signal at three (or more) hydrophones, the “time difference of arrival” (TDOA) for each pair of hydrophones can be calculated, yielding a set of hyperbolae describing constant TDOA trajectories. The point where all three hyperbolae intersect yields the transmitter location. Mathematically, the hyperbolic equation system can be described as:

$$\begin{aligned}
\sqrt{(x-x_1)^2+(y-y_1)^2} - \sqrt{(x-x_2)^2+(y-y_2)^2} &= c\Delta t_{(1,2)}\\
\sqrt{(x-x_1)^2+(y-y_1)^2} - \sqrt{(x-x_3)^2+(y-y_3)^2} &= c\Delta t_{(1,3)}\\
\sqrt{(x-x_2)^2+(y-y_2)^2} - \sqrt{(x-x_3)^2+(y-y_3)^2} &= c\Delta t_{(2,3)}
\end{aligned}$$

This system of non-linear equations can be solved for x and y (transmitter location) using various methods. According to the manufacturer, the deployed system (Map_600; Lotek Wireless inc.) used “a proprietary algorithm that provides excellent solution stability and computational efficiency, and belonging to the general class of methods employed by most GPS receivers”.

Regardless of applied technology, telemetry tagging protocols typically entail that a transmitter is attached externally, inserted intragastrically or surgically implanted into the peritoneal cavity of the fish (Lucas & Baras 2000; Bridger & Booth 2003). A standard assumption in telemetry studies is that the tagging procedure, the presence of the transmitter and the tracking protocol does not significantly alter fish behaviour, physiology, growth, survival etc. after a given period of time (see Bridger & Booth 2003 for discussion); i.e. that the 'observer effect' is negligible. This was also assumed to be the case in the present work. Although inconclusive, data from the present work support this, as the vast majority of tagged fish remained alive throughout the transmitter battery lifetime. Additionally, occasional recaptures of tagged individuals showed successful healing of incision wounds with no signs of infections and the fish were seemingly in good condition. Of the three species studied, roach was probably most susceptible to being affected by the tagging protocol. However, Jepsen & Berg (2002) report that roach equipped with radio transmitters with a trailing antenna seemingly behaved naturally and Skov et al. (2005) found no adverse effects on roach body condition from tagging with PIT tags. All fish used in the present work were tagged by surgical implantation of the transmitters following the standard protocol given in Jepsen et al. (2002); details are given in the manuscripts. Surgical implantation is generally recommended for studies of extended duration in which entanglement of externally attached transmitters is a concern (Bridger & Booth 2003). All surgical implantations were conducted by trained personnel with several years of expertise to minimize detrimental effects (Cooke et al. 2011).

Study site selection

Lake Gosmer was chosen as study site from a number of candidate lakes based on careful research and consideration. As APTSs are based on acoustic signals, study site selection was a trade-off between system performance and ecologically interesting features. For instance, habitat complexity (e.g. submerged macrophytes and intricate bathymetry) would be interesting study site features from a biological perspective but would severely impede system performance from increased signal attenuation, obstruction and reflection. Additionally, a larger study site would have been desirable for some research questions but based on conducted range tests, this would severely affect system performance due to detection range limitations. Finally, presence of self-sustained fish communities, landowner cooperativeness and control of public access had to be considered. Lake Gosmer was chosen as the best compromise between these factors, although the habitat complexity was relatively low and the area relatively small. However, these disadvantages were compensated by a near optimal bathymetry to optimize system performance, i.e. an overall concave bottom profile ('bathtub shaped') without protruding features that could acoustically shadow the hydrophones. Additionally, submerged macrophytes were virtually absent (except a small bed of water lilies, *Nuphar lutea*) and there was no public access to the lake. Narrow and dense stands of emergent macrophytes (bulrush; *Typha latifolia*) covered much of the shoreline. Finally, the lake contained a self-sustained fish community consisting of pike, perch and roach as well as a small number of stocked eel.

Study system assessment

The APTS used in the present work was a MAP_600 (Lotek Wireless Inc., Newmarket, Ontario, Canada). Preliminary test and calibration of the system setup revealed that, although it was capable of high resolution tracking of transmitters, the quality of the raw data was compromised by spurious outliers originating from numerical instability of the positioning algorithm used in the proprietary software (Niezgoda et al. 2002). Filtering based on proprietary metrics (dilution of precision (DOP), condition number (CN) and reliability number (RN); Niezgoda et al. 2002) removed the most erroneous of these. However, filtering based solely on these metrics was unable to remove less obvious erroneous positions. Therefore, to objectively reduce the influence of these remaining outliers, we processed all raw positions using a custom-made algorithm based on a Hidden Markov Model yielding the most probable track of each transmitter (Pedersen et al. 2011a; Pedersen et al. 2011b). In short, the Hidden Markov Model estimated a Gaussian two-dimensional probability density function for each registration using information from the focal as well as prior and following observations. From this probability density function the most probable position was obtained. All analyses of fish behaviour in the present work are based on these most probable positions (Fig. 3).

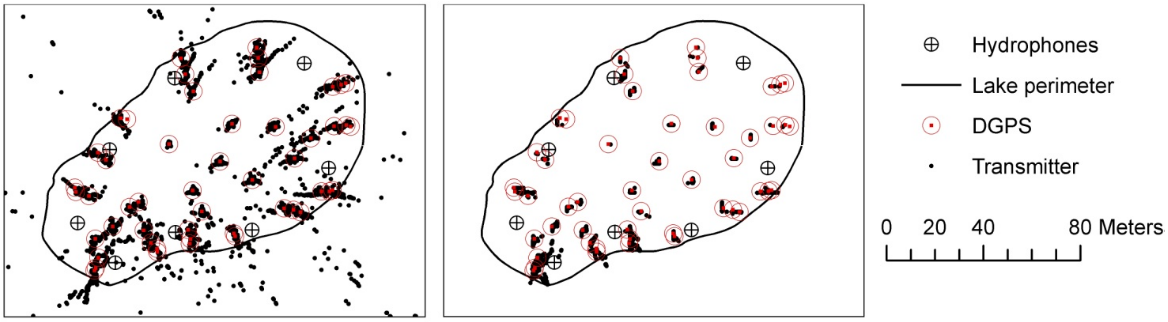


Fig. 3. Estimated positions of stationary transmitters before (left) and after (right) processing the data with the Hidden Markov Model. Each red circle indicates a stationary trial with two or three transmitters deployed in the circle centre (true position surveyed using DGPS); black dots indicate positions calculated by the ATPS. See the main text and ms/I/ for further info.

Previous studies utilizing comparable APTSs often report "sub-meter accuracy" of the systems used (e.g. Cote et al. 2002; Cote et al. 2003; Hanson et al. 2007a; Hasler et al. 2009; O'Connor et al. 2010). However, systematic whole-study-site tests of these claims have not been published. As the APTS deployed in Lake Gosmer constituted a fundamental part of the methodology applied in the present work, we designed a study to assess the performance of the system (ms/I/). The study was done in collaboration with the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB, Berlin, Germany) which allowed the inclusion of two APTS setups: the APTS deployed in Lake Gosmer and a comparable APTS deployed in a larger lake (Kleiner Döllnsee, Germany, area 24 ha). In both lakes a series of stationary tests were conducted following a standardized protocol. In each test, transmitters were moored at known positions (surveyed using a differential gps unit to ± 0.2 m) for a time period allowing the transmitters to emit several hundred signals. The transmitters were deployed to mimic potential positions of fish, by attaching them at different depths to a line held in place by a heavy sink and kept vertical by a float. The habitat structure and complexity at sites of deployment were categorized based on depth and type and quantity of the vegetation in immediate vicinity of the transmitter. In Lake Gosmer, the following habitat categories were present: dense emergent macrophytes, emergent macrophytes, shallow open water and deep open water. System accuracy and precision within each habitat category was estimated from these tests (Fig. 3). Additionally, to facilitate an evaluation of system performance for moving fish, a number of tow tests were conducted (Fig. 4). During these, three transmitters attached to a solid vertical rod were sailed around the lake following a linear to curvilinear path to mimic the trajectories of swimming fish. The study revealed that, although the quality of the data was dependent on habitat type and complexity, the APTS deployed in Lake Gosmer yielded data with good spatial accuracy and precision and thus constituted a potent tool for studying fish behaviour in natural settings; in effect, the deployed APTS turned Lake Gosmer into a "large-scale monitored aquarium" providing detailed information on the volitional behaviour of the tagged fish in their natal environment.

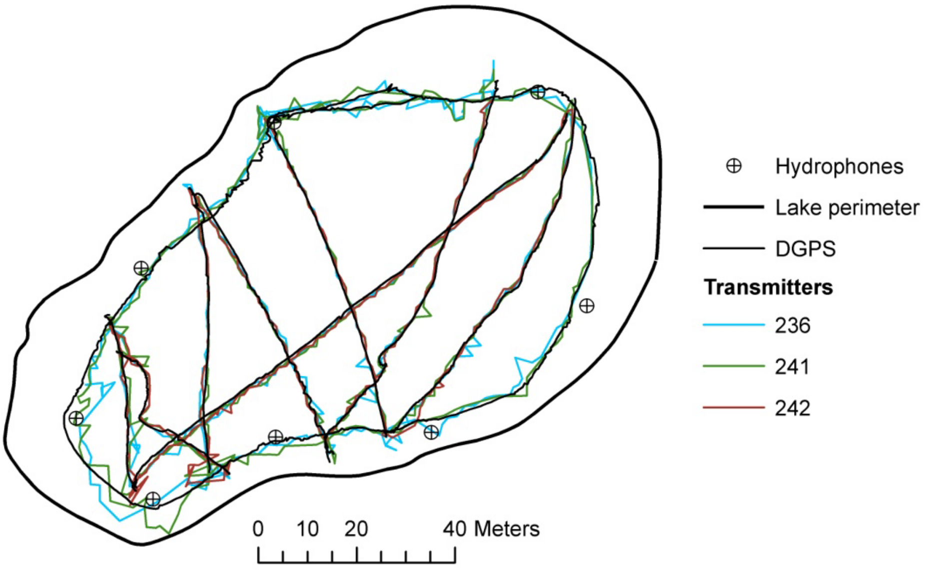


Fig. 4. Subset of the results from tow tests using three transmitters. The true trajectory of the boat is given by thin black line (DGPS); ATPS calculated trajectories of the transmitters (236, 241 and 242) are given by coloured lines. Transmitter 242 was not used in the circular tow encircling the lake. See the main text and ms/I/ for further info.

Observation frequency

Animal travel distance is an important ecological variable and can be used as a metric to describe overall animal behaviour and energetic expenditure. However, when quantified as point-to-point distances by means of positional telemetry techniques, the observed value will inevitably underestimate the true value (given that obtained positions are accurate). The degree of underestimation is dependent on observation frequency, movement velocity and path tortuosity (Hanson et al. 2007a; Rowcliffe et al. 2012). Although the APTS enabled a significant increase in temporal resolution compared to traditional positional telemetry, the effect of sampling frequency was still relevant. In acoustically based telemetry, the successful registration of an emitted signal is influenced by a probabilistic component encompassing several factors such as signal attenuation, signal obstruction and multipath propagation caused by signal reflection. Consequently, as found and discussed in ms/I/, the achievable observation frequency depended on both transmitter burst interval and the complexity of the habitat surrounding the transmitter. For instance, the observation frequency of transmitters was reduced considerably when positioned deep within dense emergent macrophytes and within dense submerged macrophytes (present in Kleiner Döllnsee). Given the habitat distribution (virtually no submerged macrophytes) and bathymetry of Lake Gosmer, it seems plausible that the only areas causing severe reduction in detection probability were within the narrow stands of dense emergent machrophytes along the shore. This was confirmed by stationary tests (ms/I/)

and by visual inspection of the complete data set from tagged fish (data not presented). As overall habitat complexity in Lake Gosmer was relatively low, these macrophytes could obviously have constituted an important habitat for the fish. However, based on obtained data it was presumed that the stands of emergent macrophytes primarily were used by pike as shelter in which the pike remained stationary; i.e. movement within the emergent macrophytes was presumed to be negligible. This presumption was based on several findings. In general, we achieved relatively high observation efficiency (i.e. number of obtained observations relative to number of potential observations) for both perch and roach and they were found to primarily reside in the open water habitats. This strongly indicates that neither perch nor roach utilised the emergent macrophytes to any significant extent. For pike, the obtained data was less conclusive. Some individuals yielded high observation efficiencies and were found to primarily reside in or frequently visit the open water habitats (typically the larger individuals). From this it was assumed that pike with lower observation efficiencies spend more time within the emergent macrophytes during which their behaviour for obvious reasons remained unknown. However, pike is generally considered a sit-and-wait predator with low overall swimming activity (Eklov & Diehl 1994; Craig 1996). Moreover, Kobler et al. (2009) found individuals specifically grouped as “reed selectors” (i.e. individuals that primarily resided in reeds) to be basically inactive in terms of movement activity. In conclusion, to paraphrase the above stated presumption, it was presumed that the APTS in Lake Gosmer was able to detect tagged fish outside the emergent macrophytes and that fish residing within the dense emergent macrophytes primarily remained stationary.

Summary | Methodology

The feasibility of the APTS deployed in Lake Gosmer to study fish behaviour was assessed and validated. The assessment study (ms/I/) confirmed that the system was capable of yielding positional data with high precision and accuracy from both stationary and moving transmitters given that appropriate processing of the raw data was applied. Additionally, the study signified the importance of careful study site selection as habitat complexity can influence system performance. Considering the bathymetry of and overall habitat distribution in Lake Gosmer, it was argued that the data obtained during the course of the present work was representative of the actual positions of tagged fish to warrant the conducted studies and inferences made therein.

Environmental factors affecting fish behaviour

A vast array of environmental factors is generally acknowledged to influence fish behaviour: e.g. temperature, turbidity, oxygen concentration, barometric pressure, lunar phase and photoperiod (Pitcher 1993). Some of these factors vary in a predictable manner (e.g. photoperiod and water temperature) and many species utilize this to time and/or synchronize distinct behavioural patterns such as spawning and migration (Lucas & Baras 2001). This type of behavioural response to external stimuli often includes a genetic component (Liedvogel et al. 2011) and constitute a significant milestone in the life history of the individual fish (Harden Jones 1968). However, the behavioural response to changes in environmental factors can be more subtle including temporarily altered activity levels, habitat utilization and feeding behaviour (Pitcher 1993; Lucas & Baras 2001).

A common attribute for most of these factors is that their isolated effect on fish behaviour, in practice, only can be studied in laboratory or small scale meso-cosmos experiments. For example, the isolated effects of water temperature, turbidity or light levels on volitional behaviour can be studied by controlling and experimentally changing their levels in a laboratory whereas that would be virtually impossible in natural systems. Therefore, studies on the effects of environmental factors in natural settings often rely on the natural variation and fluctuation that occurs during a given timeframe. However, as most of these factors co-vary to some extent (e.g. temperature and photoperiod) any detected behavioural response will be a response to changes in the suite of factors and not just a single one of them. Additionally, as natural changes in, for instance, photoperiod occur on a seasonal time scale, internal factors such as degree of gonad development and associated hormone levels will potentially co-vary with the focal environmental factor, further complicating analysis of the isolated effect of the environmental factors. In combination with the challenges inherent in extrapolating from laboratory to nature, this may lead to erroneous predictions regarding fish behaviour in natural settings. A potential example is the activity of lentic fish communities during winter, which is discussed below.

Fish communities under ice – dynamic or static?

As discussed in Salonen et al. (2009) it has been commonly believed that biologic activity, including the fish component, in temperate lentic ecosystems virtually was put on hold during winter as a result of cold water and low light. This notion of a static fish community was most probably based more on indirect evidence (e.g. laboratory findings and studies on lower trophic levels) than actual observations on fish behaviour *per se*. A common finding in studies on the isolated effect of water temperature is a clear positive correlation between temperature and fish activity up to a given optimum (Casselman 1978; Scherer & Harrison 1988; Castonguay & Cyr 1998). These results are expected from an energetic perspective as speed of biochemical processes and thus the amount of energy available to e.g. swimming activity is highly temperature dependent (Fry 1971). Additionally, overall activity of visually oriented fish would expectedly be positively correlated with light levels (Stoner 2004) as have been found in e.g. whitefish (*Coregonus clupeaformis*; Scherer & Harrison 1988) and brook trout (*Salvelinus fontinalis*; Marchand et al. 2003). Finally, primary production and consequently zooplankton biomass are known to be very low during winter in cold and ice covered lakes (Sommer 1996). A natural conclusion from the combination of these findings is that lentic fish communities probably remain very inactive and basically on pause during the winter. In turn, this assumption, in combination with the logistic and methodological challenges inherent in winter field studies, has resulted in relatively few studies on winter fish behaviour compared to the number of behavioural studies during summer (Salonen et al. 2009) (biologists' sense of comfort may also have been an influential factor; personal observation). However, recent studies suggest that lentic fish communities are not static during winter (e.g. Jurvelius & Marjomaki 2008; Salonen et al. 2009; Amundsen & Knudsen 2009).

Pike is an example of a lentic species that seemingly is "understudied" in terms of winter biology. Pike is the natural apex predator in many Northern Hemisphere lakes (Craig 1996) and it is well documented that pike profoundly can affect the prey fish community through both predation (Byström et al. 2007) and more subtle non-consumptive effects such as changes in prey fish morphology (Brönmark & Miner 1992), altered behaviour (Jacobsen & Berg 1998) and habitat utilization (Jacobsen & Perrow 1998; Skov et al. 2011). Furthermore, through cascading top-down effects, mediated by impacting the prey fish community, pike can influence the properties of the local ecosystem such as nutrient cycles and levels and, thus, water turbidity (Prejs et al. 1994; Berg et al. 1997, but see Skov & Nilsson 2007). Additionally, pike is an important species for recreational fisheries (Arlinghaus & Mehner 2004). In spite of the importance of this species, relatively little is known about the winter biology of pike.

Pike swimming activity has been shown to be highly dependent on water temperature with basically no activity below six degrees Celsius (Casselman 1978). Additionally, pike are often considered to be an ambush predator (Craig 1996). Combined with the general notion of static lentic ecosystems, this could lead to the expectation that pike remain stationary and inactive during winter. Previous field studies comparing pike activity between seasons are inconclusive as the findings range from decreased to elevated activity when comparing winter and summer (e.g. Diana et al. 1977; Cook & Bergersen 1988; Jepsen et al. 2001; Kobler et al. 2008). However, as a consequence of the applied methodologies, these findings are based on data with a rather coarse temporal and spatial resolution. Whereas long distance displacements could be detected using the methods applied in these studies, they were not able to register any small scale movements or short duration swimming bouts which can constitute a significant part of overall pike activity (Beaumont et al. 2005).

Through the development of APTSs some of the challenges in conducting studies on fish winter behaviour have been surmounted. Furthermore, the spatial and temporal resolution enables nearly complete coverage of the tagged fish. In ms/II/ we utilized these benefits to obtain time-series on individual pike behaviour that to our knowledge is unprecedented. We showed that pike swimming activity can be at surprisingly high levels and remain relatively constant from late summer through winter. Furthermore, we confirmed the diel patterns of activity presented in previous studies (e.g. Diana 1980; Beaumont et al. 2005). Additionally, we found that these patterns correlated with times of sunrise and sunset and that the seasonal changes in these patterns followed the seasonal progression in photoperiod. The study suggests that, as the apex predator in the lake remained active during winter, it is reasonable to suspect that the structuring effect of pike on the prey fish community might also be present during the cold months. This is in accordance with recent studies showing that prey fish communities can react to temporal changes in the trade-off between food availability and predation risk by undertaking partial migrations to predation refuges (Brönmark et al. 2010; Skov et al. 2011). Skov and colleagues found that the major factor influencing the individual migration propensity of prey fish during winter was individual predation risk from pike (Skov et al. 2011). This supports the suggestion of a structuring effect of pike on the prey fish community during winter.

Summary | Environmental factors affecting fish behaviour

The knowledge on biologic activity of the fish component in lentic ecosystems during winter is relatively limited. In ms/II/ we present evidence that the apex predator in these systems can remain surprisingly active throughout the seasons from summer through winter. Consequently, it is suggested that the structuring effect exerted by pike on the prey fish communities can be present during winter. Overall, the findings add to the mounting evidence that lentic fish communities are more dynamic during winter than previously thought.

Human activities affecting fish behaviour

Many outdoor recreational activities, e.g. bathing/swimming, boating, kayaking and fishing, involve some degree of interplay with natural water resources. These activities affect the natural ecosystem to a variable degree with potential effects on wildlife and vegetation. All the activities may disturb wildlife from various types of stimuli ranging from visual cues, over indirectly physical stimuli (e.g. waves and sound) to direct physical contact. Animals have evolved instinctive anti-predator behavioural responses to generalized threatening stimuli such as those originating from human activities (Frid & Dill 2002). Potential anti-predator behavioural changes include increased vigilance (Lima & Bednekoff 1999) and habitat shifts (Werner & Hall 1988). In turn, these non-lethal effects may influence the fitness of the individual animal as well as the population dynamics (Lewin et al. 2006).

Recreational angling is a popular outdoor activity in many countries. Whether performed from the shore or from a boat, angling will inevitably cause some level of disturbance. The degree of disturbance will naturally be linked to type of angling and behaviour of the angler. Even if the ultimate goal of angling, i.e. hooking, catching and possibly releasing a fish, fails, the activity of angling *per se* can influence fish behaviour in various ways through a range of stimuli. Whereas the direct effect of contact between fish and angler produces a clear and immediate behavioural response, the effects of indirect stimuli can be more subtle. For instance, when a fish is hooked by an angler, it displays a very direct and detectable response whereas the effect of water movement or noise produced by an approaching boat can be a minor change in swimming activity that can be hard to identify. The following will focus on fish behavioural response to 1) stimuli induced by boat based angling and 2) catch and release.

Boating and angling from boat

Boating, whether in relation to angling or not, produces a range of stimuli that potentially affect fish behaviour. Although hard to isolate from other disturbance factors (e.g. visual stimuli and waves), underwater noise produced from an outboard engine is most probable a major constituent of the collective disturbance assemblage from this type of activity. From laboratory studies it is known that anthropogenic noise can affect the physiological homeostasis of fish through changes in cortisol levels and other biochemical parameters (Santulli et al. 1999; Smith et al. 2004; Wysocki & Ladich 2005; Wysocki et al. 2006). Additionally, different means of small boat propulsion methods (paddle, electric motor and combustion engine) all affect cardiovascular performance in largemouth bass (*Micropterus salmoides*) but with different magnitudes (Graham & Cooke 2008). By recoding underwater boat noise in the field and playing it back in an experimental tank, Boussard (1981) investigated the response of roach and rudd (*Scardinius erythrophthalmus*) to the sound stimuli. The typical response was reported as a direct movement to the opposite end of the tank relative to the location of sound emission. Additionally, the fish responded more readily to the initial stimulus than to subsequent bursts indicating a habituation effect.

From marine field studies it is evident that large fishing and research vessels have the capacity to provoke short-term behavioural changes in several pelagic fish species (e.g. Soria et al. 1996; Vabø et al. 2002). For instance, active avoidance reactions to fishing vessels have been found in cod (Handegard et al. 2003) and herring (Vabø et al. 2002). However, field studies on fish behavioural response to the apparently benign human disturbances caused by angling and boating are scarce. Mueller (1980) found that small recreational boats moving at slow speeds can alter fish behaviour as suggested by the laboratory findings of Graham & Cooke (2008) showing that paddle strokes can affect cardiovascular performance. In a recent study on the non-lethal effects of angling, Klefoth et al. (2011) found that in response to the direct effect of catch-and-release, pike reduced swimming activity and suffered reduced growth rates. Additionally, by analysing the movement of pike that were not caught by anglers during the study, Klefoth et al. (2011) found no behavioural response to the indirect disturbance caused by the angling protocol (however, these findings are to some degree confounded by protocol issues as discussed below).

In ms/III/ we present field data on behavioural responses of free swimming fish to angling related disturbances as warranted by Graham & Cooke (2008) and Slabbekoorn et al. (2010). The study was carried out in Lake Gosmer which was equipped with an APTS (described above or see ms/I/). One of the major benefits of APTS for this study was the automatic tracking of the tagged fish that renders manual tracking redundant. This allows the lake and tagged fish to be left completely undisturbed by human activities for extended periods, thereby providing a control situation in experiments designed to quantify the effects of disturbances (c.f. the overlap in treatment and data acquiring protocols in Klefoth et al. (2011) discussed below). To model typical boat based angling behaviour we used a small fibreglass boat powered by a 4 hp outboard combustion engine driven slowly around the lake following a standardized protocol. In short, the study focused on the behavioural response of roach, perch and pike to three different levels of disturbance (treatments): no disturbance (control), disturbance by boating and disturbance by boating and angling (see ms/III/ for details). Through the APTS we obtained detailed field data on the immediate and short term response of the three species to this type of human disturbance. As behavioural response variable we used individual swimming speed calculated as Euclidian distance between two consecutive positions divided by time (see ms/III/ for further details). All three species seemingly responded to the disturbance protocol, but the responses were species specific. Whereas roach and perch increased activity, the results for pike are less conclusive but indicate that pike responded by reducing their overall activity. Both roach and perch displayed maximum response immediately which then tapered off in the following hours, which is in accordance with Boussard (1981) who found a stronger response to the initial stimuli compared to subsequent. This might indicate a habituation effect where the fish no longer respond as much to or basically ignore the stimuli. However, although the activity returns towards control levels, the fish might still be influenced by the stimuli and their apparently normal activity level might represent behaviour differing from their normal undisturbed behaviour. An in-depth analysis of this would require visual observations of the individual fish detailed behaviour (e.g. foraging and hiding) thereby enabling a time-budget analysis (e.g. Picciulin et al. 2010). Even though an APTS is unable to quantify fish behaviour *per se* the spatial data produced by the deployed APTS can be indicative of altered behaviour. For instance, the spatial distributions of roach during control and treatment days suggest that the distribution of roach was constricted during the disturbances (Fig. 5). Additionally, a comparison of spatial distributions for the individual perch (area of individual 90 % kernel density) for control and treatment days revealed that the individual distributions expanded in response to the disturbances (Fig. 6; paired t-test, n = 22, p < 0.01; preliminary results). Thus, the disturbance protocol affects both fish activity and their spatial distributions.

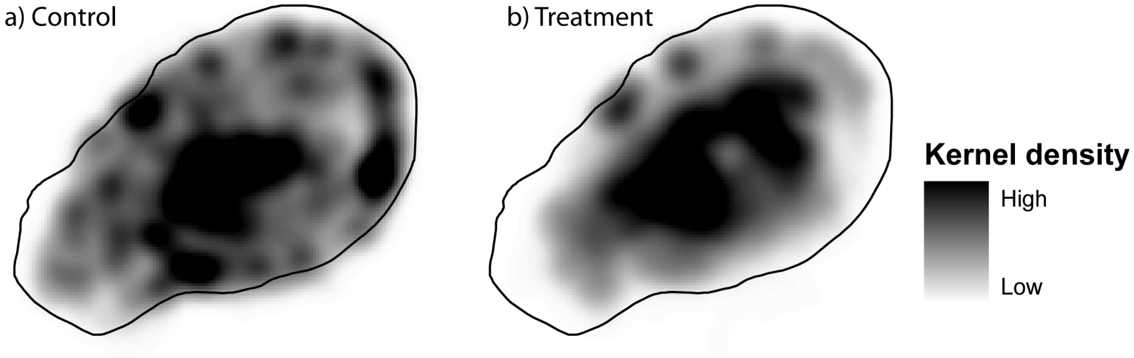


Fig. 5. Kernel density plots of the habitat utilisation of roach during control days (a; n = 10) and days with disturbance (b; n = 10). The roach seemingly responded to the disturbance by aggregating in the centre of the lake.

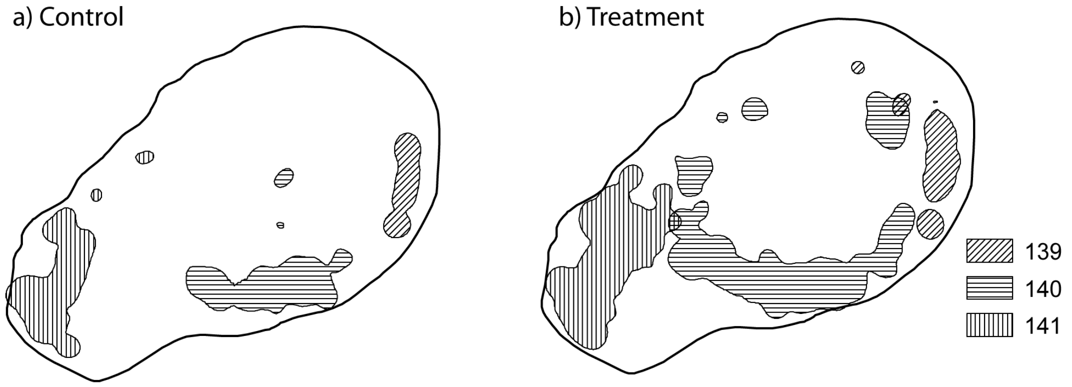


Fig. 6. Selected examples (three perch) of individual 90% kernel density utilisation plots during days without (a; n = 10) and with disturbance (b; n = 10). During days of treatment the tagged perch utilized a larger area than during control days (paired t-test, n = 22, p < 0.01).

Roach and perch are both potential prey for pike. As such, the spatial distribution of these species will, at least in part, be the result of a trade-off between foraging opportunity and the risk of being predated. It is thus conceivable that the undisturbed distribution of both prey species to some extent represent the optimal compromise for the individual prey fish (Lima & Dill 1990). Therefore, the alteration of prey fish distribution induced by the disturbance protocols might result in the roach and perch experiencing sub-optimal conditions, both in terms of available food and predation risk. Additionally, the induced increase in activity levels of roach and perch will *ceteris paribus* increase the probability of pike-prey encounters. However, the results from ms/III/ suggest that pike in Lake Gosmer (if anything) reduced their activity in response to the disturbances, potentially counteracting the increase in pike-prey encounter probability. Both Klefoth et al. (2011) and ms/IV/ found reduced activity in pike exposed to direct stimuli through capture and release. Though the stimuli used by Klefoth and colleagues are not directly comparable to those used in ms/III/ and ms/IV/, they support that pike display an innate behavioural response to disturbances (i.e. reduction of activity) as speculated by Klefoth et al. (2011). In contrast to ms/III/, Klefoth et al. (2011) found no behavioural response to angling related indirect disturbances. However, to quantify pike movement activity, Klefoth and colleagues utilized a manual tracking protocol that potentially caused the same type and amount of stimuli as the indirect angling derived disturbances they hypothesized could affect pike movement activity; boats and motors used in both tracking and fishing protocols were the same. As a side note, this overlap between treatment and data acquiring protocols exemplifies one of the potential shortcomings in telemetry relying on manual tracking: the tracking activity per se can potentially affect the volitional behaviour of the focal fish. The reduction of pike activity might reflect changes in pike behaviour resulting in a relaxation of the predation pressure on the roach and perch during the disturbances. If disturbance frequency and intensity are high, this could lead to decoupling of the predator-prey interactions involved in structuring the lake ecosystem with potential effects on lower trophic levels (Carpenter & Kitchell 1993). Although interesting, the intricate interplay between anthropogenic disturbances and interspecific interactions was beyond the scope of this work but could be addressed in future studies.

Catch and Release (C&R)

Fisheries management based on the catch and release (C&R) principle has become quite popular in recent years as a mean to alleviate the potential conflict between high angling pressure on popular species and limited natural resources (Arlinghaus et al. 2007). A crucial assumption underlining C&R based management is that survival and fitness of the caught and released fish are not severely affected by the event. Multiple studies on C&R angling have documented post-release effects on behaviour (Pepperell & Davis 1999; Cooke et al. 2000; Thorstad et al. 2004; Thorstad et al. 2008; Thompson et al. 2008; Arlinghaus et al. 2008a) and physiological homeostasis (Gustaveson et al. 1991; Kieffer 2000; Cooke et al. 2002; Arlinghaus et al. 2009) which in turn can affect e.g. predator avoidance (Cooke & Philipp 2004), growth

(Siepker et al. 2006; Cline et al. 2012) and reproductive success (Ostrand et al. 2004). For exhaustive discussions of currently available knowledge on effects of C&R angling see Arlinghaus et al. (2007) and Lewin et al. (2006).

Pike is a very popular target species for recreational fishing in both Europe and North America (Pierce et al. 1995; Fayram 2003; Arlinghaus & Mehner 2004). To sustain viable population sizes and/or increase the chances of trophy fish, C&R is implemented in many pike fisheries either as mandatory or voluntary (Pierce et al. 1995; Paukert et al. 2001; Arlinghaus & Mehner 2004). However, despite intensions of the opposite in the C&R rationale some mortality directly linked to the capture events is unavoidable. Handling time, which can be substantial as "anglers may fear personal injury from this toothy predator" (quote Arlinghaus et al. 2009), and hooking position are important factors in C&R related mortality in pike (DuBois et al. 1994). Mortality rates of pike related to C&R angling have been reported to range from zero and up to 33% (reviewed in Arlinghaus et al. 2008b). Besides post-release mortality, C&R is known to inflict several sub-lethal effects on pike. For instance, Colotelo & Cooke (2011) found extensive damage to the epithelium of pike as a result of some non-optimal C&R practices. Furthermore, studies have found that an angling event induces a physiological response in terms of altered blood parameter levels (Schwalme & Mackay 1985a; Schwalme & Mackay 1985b; Arlinghaus et al. 2009). A few studies give indications regarding pike post-release behaviour. From these there seems to be consensus that pike respond to the C&R event by temporarily decreasing their activity (Klefoth et al. 2008; Arlinghaus et al. 2009; Klefoth et al. 2011). Additionally, Kuparinen et al. (2010) found that past fishing effort influence short term angling catch rates, which they ascribed to a behavioural response.

In ms/IV/ we present the first study utilizing an APTS to monitor the detailed behavioural response of pike to C&R events. The novelty of this study, besides the level of detail, is the inclusion of previously tagged but uncaught pike functioning as a control group undisturbed by a tracking protocol. We attempted to isolate the effects of the C&R event by comparing the behaviour before and after C&R and accounting for population wide effects using the control fish. The main finding was that caught and released pike generally displayed lower activity levels after relative to before the C&R event. This effect was transitory and could not be detected after two days. Additionally, the decrease in activity was positively correlated with water temperature, i.e. higher temperature resulted in a larger reduction in activity. Although the main finding is in line with previous studies (e.g. Klefoth et al. 2008; Arlinghaus et al. 2009; Klefoth et al. 2011) the detailed data on movement activity indicate that the response is more complex than a simple general decrease in activity. For instance, from figure 2 in ms/IV/ it is evident that some individuals react with an immediate activity cessation (e.g. third from the top) whereas others respond by a period of increased activity followed by an activity reduction (e.g. second from the top).

Summary | Human activities affecting fish behaviour

The findings from ms/III/ and ms/IV/ add to the mounting evidence that recreational boating and angling can affect fish behaviour in several sub-lethal ways with potential implications for individual survival and fitness. From a management perspective this type of information can be valuable as it allows the formulation of management plans based on scientific knowledge regarding the response to human activities. For instance, establishment of areas with restricted or no access could be considered in larger lakes with high intensity of human activities.

Inter-individual variability

Inter-individual variation in animal behaviour has been acknowledged as being an important ecological and evolutionary characteristic of wild populations (Sih et al. 2004). However, the mechanisms involved in maintaining such consistent differences on both individual and population levels are not fully understood. The following section explores potentially explanatory relations between inter-individual variation in behaviour, metabolism and morphology through an inter-disciplinary approach. This was done by combining information on inter-individual variation in morphology, boldness and metabolism obtained in the laboratory with volitional behaviour in natural settings of the same individuals.

Inter-individual variability in behaviour

Field studies on fish behaviour often reveal a large inter-individual variation in many behavioural traits such as home range size, movement activity and swimming speed. A part of this variation can often be explained by simple measures such as body size (encompassing potential ontogenetic shifts in behaviour) and condition factor. However, even when correcting for this, the residual variation in behaviour is often considerable as noted in Jepsen et al. (2001): ”*There were large behavioural differences between fish in the lake and in the reservoir and even more variation between individuals within each population*”. In many behavioural studies, this residual variation is considered a nuisance and is often ignored as natural variation or noise complicating an extrapolation from tagged individuals to a population level (Careau et al. 2008). However, in recent years, these differences in behaviour of otherwise similar individuals have gained increased focus through the emergence of concepts such as behavioural syndromes, animal personalities (e.g. Sih et al. 2004; Bell 2007) and behavioural reaction norms (Dingemanse et al. 2010). These concepts are based on the recognition that individual animals often display some degree of behavioural consistency across different contexts (Fig. 7). Understanding the cause and effect of such consistent differences in behaviour is fundamental, as natural selection ultimately operates on an individual level. However, at present empirical data on free ranging animals with pre-quantified behavioural tendencies is scarce. Therefore, as part of the present work, we initiated a study focusing on the ecological relevance of laboratory derived measures by linking behavioural consistency in a lab experiment with volitional behaviour in the field (Box 2). Unfortunately, this study was unsuccessful due to external factors and the time frame of the present work did not allow for further pursue of this interesting and burgeoning research area. However, future studies focusing on the ecological relevance of animal personalities are warranted.

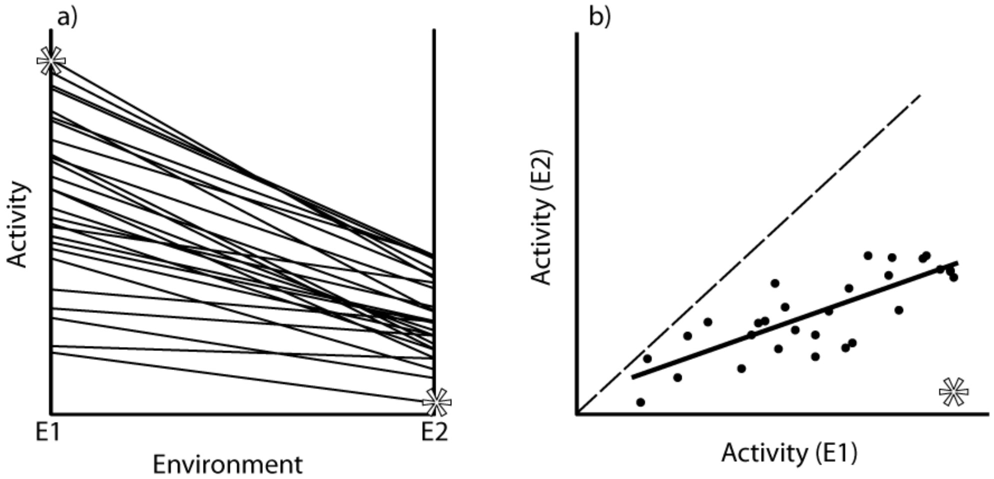
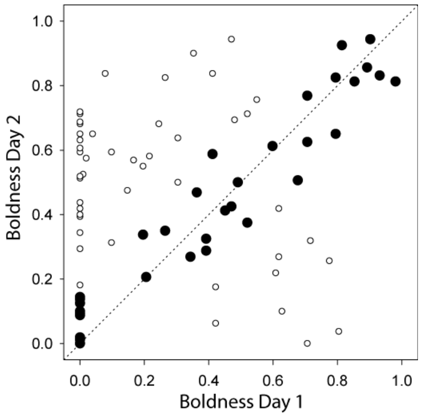


Fig. 7. Conceptual representation of consistent differences in individual behaviour between environments (E1 and E2): plasticity plot (a) and correlation between environments (b). E1 and E2 represent, for instance, activity in environments without and with predators, respectively. The optimum activity for each environment is indicated by the asterisks. Each individual is represented by a line in (a) and a dot in (b). Solid and dashed lines in (b) represent the regression through the dots and a 1:1 reference line, respectively. Redrawn from Sih et al (2004).

BOX 2

Bold roach – lab effect or real life phenomenon?

A study was designed to test the assumption that individual boldness measured in the lab entails behavioural differences in natural settings. Eighty five roach were captured and screened for behavioural consistency using a refuge emergence protocol (Brown et al. 2007). Chapman et al. 2011 used a comparable protocol to assess boldness in roach and found behaviour to be consistent and repeatable. Individual roach were placed inside a closed refuge box and left to acclimatise for 15 minutes. A door was lifted and time for fish to fully emerge was used as an index of boldness. Each fish were assessed on two separate days to identify individuals showing consistent boldness. Based on these data we selected 39 individuals that were subsequently tagged with acoustic transmitters (Lotek MAP 6_2; burst interval 15 sec) and released in the Lake Gosmer to quantify their behaviour in a natural environment. However, due to an unexpectedly high transmitter failure rate (33 of the 39 malfunctioned) no useful data on the field behaviour of these fish were obtained.



Selection of roach with repeatable boldness. Each point represents boldness scores of an individual roach from two separate days. Roach with consistent boldness (filled circles) were selected for tagging and release in Lake Gosmer.

Inter-individual variability in metabolism

Researchers within the field of fish physiology are often faced with a situation comparable to behavioural biologists. Measures on metabolic rates most often contain inter-individual variation; also when studying animals of similar size (or correcting for size differences) and even when using siblings (Metcalfe et al. 1995). Similar to the observed variation in behaviour, inter-individual physiological variability have traditionally been ignored as random noise (Bennet 1987). However, analogous to the behavioural consistency discussed above, metabolic rates are found to be repeatable and consistent (Cutts et al. 2001; Maciak & Konarzewski 2010; Norin & Malte 2011) and may reflect biologically important inter-individual differences ultimately affecting individual fitness (Burton et al. 2011). The exact causes of the inter-individual differences in metabolic rates are complex and not fully understood but studies have found evidence that factors such as maternal effects (Giesing et al. 2011) and internal organ size (albeit in mice; Konarzewski & Diamond 1995) are influential.

Linking metabolism and behaviour

As the metabolic properties of an individual define the possibilities of that individual to perform aerobic fuelled activities, metabolism and behaviour are interlinked although the mechanisms behind are complex and the direction of causality is unknown. However, a potential link between consistent individuality in metabolism and behaviour has recently been acknowledged (Careau et al. 2008; Biro & Stamps 2010; Burton et al. 2011). A mechanism proposed to facilitate this coupling is the concept of an individually sized "metabolic machinery" that on one hand enables energy output but on the other hand requires maintenance (the 'performance model' *sensu* Careau et al. 2008). Following this, individuals with relatively large machinery capable of producing more energy to fuel aerobic activities (e.g. movement, generation of somatic or gonadal tissue) are faced with a need for higher and/or more efficient energy uptake. For example, as individuals are expected to display behaviour that increase food intake rate, 'high energy' individuals should be more active and explore larger areas to sustain their metabolic machinery, given they rely on an active food search strategy. Other feeding strategies can entail different behaviours to optimize food intake such as dominance and aggression. In contrast, 'low energy' individuals will have lower amounts of available energy for activity but also have lower maintenance needs i.e. a lower need to be active. The relative fitness of each strategy will then depend on, for instance, predation pressure (Fig. 7). Environments with low predation risk, will favour individuals with high energy throughput, whereas these individuals will have a disadvantage in high risk environments. Several studies on fish behaviour have found support for the performance model in form of positive correlations between metabolic rates and e.g. positioning in schools (Killen et al. 2012), vulnerability to angling (Redpath et al. 2010), behavioural dominance (Cutts et al. 2002), risk-taking (Killen et al. 2011) and migration propensity (Lans et al. 2011). As natural environments typically are unstable and both food availability and predation pressure fluctuate, this mechanism constitutes a potential causal link between metabolism and behaviour. Furthermore, this link can potentially explain the co-existence of consistent differences in individual behaviour (i.e. behavioural syndromes). The majority of work in this field has been done in artificial laboratory settings and care should be taken when extrapolating from laboratory findings on both metabolism and behaviour to their respective relevance in nature (Blake 1991). For instance, fish behaviour in laboratory trials might not be a good predictor for fish behaviour in the wild as indicated in Klefoth et al. (2012). Furthermore, it is recognised that individual personality might affect the measurement accuracy in metabolic studies through individual differences in reactions to being confined in a respirometry chamber (Careau et al. 2008). Thus, although correlations between individual metabolism and behaviour have been found in laboratory experiments it is virtually unknown whether these correlations exist in nature.

In ms/V/ we present a study focussing on this issue by testing the hypothesis that individual metabolic properties are correlated with behaviour in natural settings. In short, we captured 23 wild perch in the study lake, tagged them with acoustic transmitters, measured standard and maximum metabolic rates (SMR and MMR) in the laboratory (see box 3) and returned them to the lake to quantify their individual behaviour. Additionally, individual scope for aerobic activity was calculated as the difference between MMR and SMR. We estimated three behavioural measures each of which was expected to correlate with the metabolic properties: overall activity, average swimming speed and maximum swimming speed. Interestingly, the data showed no indications of the correlations between metabolic rates and behaviour as predicted from the performance model. This was surprising as the species used utilizes an active search feeding strategy. However, perch are known to undergo ontogenetic shifts in prey preferences and perch in the size range used will typically be feeding on either benthic invertebrates or pelagic prey fish (or a mix of these) (Hjelm et al. 2000) and may thus have constituted functionally different groups entailing different behaviours. Therefore, metabolism-behaviour correlations may have been present within each group but this could not be tested in the present study as no information on individual feeding strategy was available. Nevertheless, the data suggests that laboratory measures of metabolism *per se* are not linked to volitional behaviour in free swimming fish in an unambiguous way.

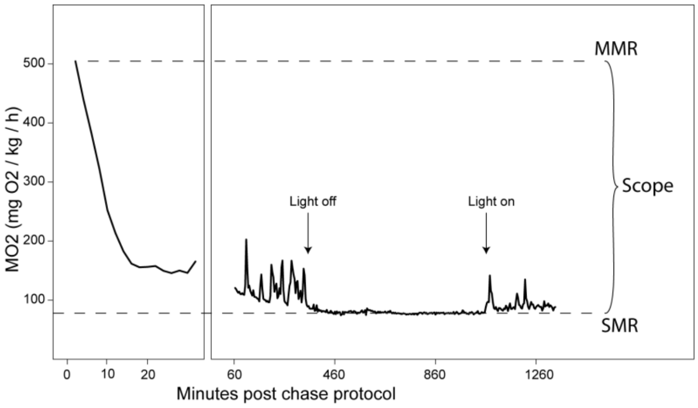
BOX 3

Measuring metabolic rates

Metabolic rates (MRs) in fish are often measured as whole-animal metabolism using the automated intermittent-flow method (e.g. Norin & Malte 2011). Individual fish are placed in a respirometry chamber immersed in an ambient tank containing fully aerated (dependent on research question) water. The chamber is connected to two separate water circuit systems generating flow through the chamber using computer controlled pumps operating alternately in a two-phased (flush and measurement) cycle. The flush phase (open circuit) is used to exchange the water in the chamber with ambient water, thereby avoiding accumulation of CO2 and other excretory products. During the measuring phase, water is recirculated in a closed circuit including a galvanic oxygen electrode measuring the oxygen tension in the water. As this is a closed circuit, fish respiration will result in continuously decreasing oxygen tensions. After correcting for oxygen solubility, atmospheric pressure and water temperature, fish oxygen consumption (i.e. metabolic rate (MR); unit: mg $O_2 * h^{-1}$) can be calculated as the slope of a regression line fitted through the oxygen tension vs. time values. To enable between-individual comparison, the obtained values are corrected for differences in mass using a scaling factor (0.8 is typically used (Clarke & Johnston 1999)) to accommodate the allometric relationship between metabolic rate and mass following the formulae: Mass specific MR = $(1/\text{fish mass})^{0.8} * \text{MR}$ (Reidy et al. 2000). Thus, mass specific MR (unit: mg $O_2 * h^{-1} * kg^{-1}$) is a measure of the MR for fish of a particular mass, typically 1 kg.

Standard MR (SMR), maximum MR (MMR) and scope for aerobic activity (SAA)

In ms/V/ we used a chase protocol (Cutts et al. 2002) to measure the maximum metabolic rate of perch. This protocol relies on the assumption that an exhausted fish is respiring at maximum possible rate. Each fish was chased individually by hand in a small tank until it no longer reacted when turned up-side-down and partially lifted out of the water. At that point the fish was quickly transferred to a respirometry chamber and the measuring phase was started immediately. The first measured MR was defined as MMR. Subsequently, the fish were left undisturbed overnight while oxygen consumption was monitored continuously. During this period the MR reached a low and stable level, typically shortly after the lights were turned off. This level was defined as SMR. SAA was calculated as the difference between MMR and SMR. The figure shows data from one of the perch used in ms/V/.



Inter-individual variability in morphology

Swimming costs is one of the major components in the energy budgets of fish (and other aquatic animals) and adaptations to reduce these costs should be evolutionary favourable. The hydrodynamics of swimming fish are complex but can be approximated by simplifications as the energy required for simple objects (e.g. a cylinder or prolate spheroid (explained below)) to move through water can be modelled relatively precisely by complex physical formulae. A key component in physical modelling of movement is resistive drag, i.e. the resistance an object is subjected to as it moves through water. Overall shape is a major determinant of drag. By considering fish a simple object (a prolate spheroid; a spheroid in which the polar axis is greater than the equatorial diameter, e.g. an American football), the effect of body shape on swimming costs has been modelled using fineness ratio as a metric of overall body shape (Blake 1983). In this case, fineness ratio is defined as the ratio between length and maximum diameter. A fineness ratio of ≈5 was found to be optimal for reducing drag according to a "simple" (quote Chung 2009) hydro-mechanical model (Blake 1983). However, using a more advanced model Chung (2009) predicts fineness ratios ≈8 (i.e. a more elongated body) to be optimal. These numbers are approximations as a fish body is more complex than a rigid prolate spheroid due to e.g. protruding, moving fins and an asymmetrical, flexible body. Furthermore, different swimming gaits will potentially warrant different values to minimize swimming costs. Thus, although the exact value is debateable, fish should have evolved body shapes with fineness ratios in the range of 5 – 8 to minimize swimming costs; this is indeed found in many species of actively swimming fish, e.g. cod and herring (Blake 1983).

Linking morphology and behaviour

Following the above paragraph and assuming that the optimal fineness ratio for a given species is constant, inter-individual variation in fineness ratio should directly influence individual swimming costs which in turn could influence behaviour. Support for this has been found in several species. For instance, Ohlberger et al. (2006) found a direct relationship between swimming costs and fineness ratio in carp (*Cyprinus carpio*) and roach. Conformingly, Boily & Magnan (2002) found that stout (i.e. relatively low fineness) individuals of yellow perch (*Perca flavescens*)experienced higher swimming costs than slender individuals. However, standard laboratory protocols used to determine swimming costs involve forced swimming against a unidirectional constant flow; a situation few fish is ever faced in nature and which relevance for free swimming fish has been questioned (Boisclair & Tang 1993). Consequently, though fineness ratio has been found to influence swimming cost, it is basically unknown whether inter-individual variability in this metric correlate with volitional behaviour of free-ranging fish. An exception is Hanson et al. (2007b) who found that body shape (measured as a PCA-axis describing features that would correlate with fineness ratio) influenced both mean swimming speeds and travelled distance of nest guarding largemouth bass. Additionally, in ms/V/ we found positive correlations between fineness ratio and both mean daily speed and maximum daily speed; i.e. slender individuals swam faster than stout individuals. Although the prospect of a pure physical explanation (i.e. the hydrodynamic effects of fineness affecting swimming costs) of parts of the individual variability is alluring, this correlation most probably entails some biological components as well. For example, fineness ratio could be related to nutritional status as 'fatter' fish of a given length will have a lower fineness ratio. Moreover, in ms/V/ we used perch whose morphology is known to be highly plastic and to correlate with both habitat structure and feeding mode (Olsson & Eklöv 2005). Generally, deep bodied and thus highly manoeuvrable perch are associated with the benthic niche, whereas slender perch are associated with pelagic feeding (Hjelm et al. 2000; Svanback & Eklöv 2004). Regardless of the causal mechanism driving the correlations between fineness and behaviour, it is interesting that this simple metric can explain significant amount of inter-individual behavioural variation.

Summary | Inter-individual variability

During the course of the present work, inter-individual variability has been omni-present as is the case in most field studies on fish behaviour. In the present work, behavioural within-individual variation was often considerably smaller than between-individual variation. Thus, individual behaviour could be considered consistent and repeatable and could therefore be analysed within the framework of behavioural syndromes. A majority of work in this burgeoning field is based on theoretical or laboratory derived findings and very few studies provide empirical data from the field. In ms/V/ we present a field study exploring some of the factors potentially linked to individual behavioural consistency. We found none of the predicted correlations between metabolic properties and behaviour. Although the data potentially were confounded by other factors enshrouding a correlation, the findings suggest that metabolism is not an overriding determinant of individual volitional behaviour. In contrast, we found morphology to be correlated with behaviour but the causal mechanism behind this is unknown and can be multifarious and complex.

Conclusions and perspectives

The present work aimed at expanding the current knowledge on fish behaviour and factors potentially affecting it. To achieve this goal, an advanced telemetry system was deployed in a small lake. Validity of data obtained using the telemetry system was assessed and found to satisfactorily facilitate studies on fish behaviour in natural settings (ms/I/).

By comparing activity levels in periods from summer through winter for two consecutive years, ms/II/ presents evidence that pike can be surprisingly active during this season. This adds to the rather limited but growing body of evidence that the fish component of lentic ecosystems is not static and inactive during winter as has previously been believed. Furthermore, ms/II/ suggests that the structuring effect of pike on lower trophic levels may be present during winter as well. This have been addressed in a subsequent study conducted in Lake Gosmer (winter 2011-2012) including roach, perch and pike, but these data have not yet been thoroughly analysed.

Manuscript ms/III/ focused on the impact of human recreational activity on fish behaviour. It was documented that angling-related boating can affect both activity level and spatial distribution of roach and perch, whereas the data on pike was inconclusive. The study indicates that disturbance from boating was the main factor affecting fish behaviour whereas the addition of angling activities did not cause increased response. To achieve a sufficiently long study period, the transmitters used in this study had relatively large burst intervals (30 sec). While adequate for the main focus of that study (i.e. is there an effect or not), this burst interval was unfortunately too large to allow further examination of the detailed response of the fish. For instance, it is likely that the magnitude of the response depends on the distance between boat/motor and fish and that the fish do not respond at all beyond a certain range. These questions could be addressed by using transmitters with lower burst intervals (e.g. 1.3 or 2.5 sec).

Management of freshwater fisheries is often based on or include catch-and-release as a remedy to allow recreational fishing while protecting vulnerable populations and/or enhance the fishery. Ms/IV/ focused on the short term behavioural response of pike to C&R-events. The general response was found to be a transitory decrease in activity of C&R pike. As no effect could be detected after two days, the findings indicate that pike are relatively resilient to C&R and, thus, that C&R can be a feasible tool in successful management of pike fisheries.

Consistent individual differences in behaviour, and the causes and consequences thereof, is a contemporary and burgeoning field of research primarily based on findings from laboratory studies. Ms/V/ presents one of the first studies attempting to bring this topic to the field through an inter-disciplinary approach. It was expected that metabolic properties and behaviour in natural settings was correlated. Although the existence of such correlations cannot be conclusively excluded, the findings suggest that metabolism is not necessarily an overriding factor determining behaviour and vice versa. Additionally, ms/V/ presents data suggesting that a simple measure of overall morphology potentially can explain a significant amount of variation in individual behaviour, although the mechanisms driving this relationship are unclear.

Overall, the application of an APTS to study fish behaviour in a small lake proved to be feasible. The approach enabled the study of detailed fish behaviour by means of geographical positions, although the exact behaviour of the focal fish (e.g. foraging, digesting) remained unknown. However, indications of physiological status of the fish and discrimination of the intricate behaviour at these positions can be obtained through the use of advanced telemetry tags measuring various important parameters. Examples include tags capable of measuring heart rate, muscle activity, tail beat frequency, overall body posture and tri-axial acceleration (see for instance Lucas et al. 1993; Cooke et al. 2004; Svendsen et al. 2005; Ohlberger et al. 2007; Donaldson et al. 2008). A combination of this type of data with the positional data obtained by an APTS could be very interesting and give further insight in virtually every aspect of fish behaviour in natural settings.

The manuscripts included in the present thesis represent only a small part of the data produced by the APTS in Lake Gosmer and potential analyses thereof. At the time of writing, the Lake Gosmer database contains nearly 50 million observations on more than 250 individual fish. These data hold great potential for further analyses of, for instance, consumptive and non-consumptive predator-prey interactions as well as detailed and long term habitat utilization and spatial distribution. Additionally, the data can potentially be analysed within the framework of important ecological models and hypothesis such as ‘ideal free distribution’ and theories on optimal movement patterns (e.g. Brownian movement, correlated random walk and Lévy walk (Viswanathan et al. 1999; Codling et al. 2008)).

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Papers I-V

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Assessing the accuracy, precision and efficiency of two automated acoustic telemetry systems covering two freshwater lakes

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Assessing the accuracy, precision and efficiency of two automated acoustic telemetry systems covering two freshwater lakes

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Abstract

Acoustic positional telemetry systems (APT) represent a relatively new approach to studying aquatic animal behaviour. System manufacturers promise high quality positional data with high temporal and spatial resolution (sub-meter accuracy). Even though APT systems have been utilized for a number of biological studies, the achievable data quality has not yet been rigorously tested. Therefore, we conducted a series of standardized tests to assess the performance of two APT systems deployed to cover two freshwater lakes of different sizes (25 ha and 1 ha). The sub-meter accuracy claim from system manufacturers were met under certain conditions, but overall system performance was found to be dependent on habitat complexity. Although species associated with complex habitats can compromise system performance, our study reveals that APT systems can provide unprecedented data on aquatic animal behaviour under natural conditions.

Introduction

Telemetry is one of the most versatile and important tools of biologist undertaking field studies on aquatic animal behaviour in natural settings. The technology can provide researchers detailed information on an individual level (Metcalfe & Arnold 1997) that would otherwise be unobtainable due to the challenges inherent in the study of underwater animals (Block 2005) and embodies a wide assemblage of technologies ranging from relatively simple Passive Integrated Transponders (PIT) to advanced data storage tags relaying collected information via satellites. Corresponding applications in the fields of fish ecology and ethology range from monitoring the migration patterns in small lakes and streams (Aarestrup et al. 2003; Svendsen et al. 2007; Skov et al. 2011) to studying the behaviour of oceanic species (Aarestrup et al. 2009; Block et al. 2001). Studies on an intermediate spatial scale, e.g. river systems and lakes, have often utilized telemetry based on either radio or acoustic signals enabling tracking of animals equipped with transmitters. Typical tracking strategies include 1) automatic passive monitoring of the presence/absence of tagged individuals covering a large area (e.g. Aarestrup et al. 2008); 2) manual active tracking enabling precise positioning of the tagged individuals (e.g. Klefoth et al. 2008; Arlinghaus et al. 2008; Kobler et al. 2009) or 3) a combination of the two (e.g. Aarestrup et al. 1999; Koed et al. 2006). Using these techniques, many aspects of fish behaviour have been studied and revealed. However, widely used methods suffer from a trade-off between spatial and temporal resolution. Whereas the passive monitoring of presence/absence of tagged individuals typically is done with high temporal resolution, it yields little or no information regarding the location, habitat choice and activity of the fish within the covered area of interest, which often extend several thousands of square meters. In contrast to this, precise positioning of tagged individuals is achievable using manual tracking (Kobler et al. 2008). However, manual tracking is labour intensive as only one individual can be positioned at a time, often resulting in relatively short continuous tracking sessions with low temporal resolution. Furthermore, as manual tracking requires active movement of the tracking personnel, often in the vicinity of the focal animal, the tracking protocol *per se* can induce altered behaviour of the focal animal.

Following the recent advent of acoustic positional telemetry (APT) systems, some limitations of traditional telemetry techniques are potentially surmounted (Niezgoda et al. 2002; Cooke et al. 2005). The APT rationale effectively minimizes the described trade-off between temporal and spatial resolution through automatic and continuous data collection and storage. In APT systems, arrays of multiple synchronized hydrophones located at fixed positions detect the acoustic signals emitted from tagged individuals and store the information. The temporal resolution of the data is thus determined by the burst interval of the used transmitters, typically sub-minute. Subsequently, acquired data are post processed to obtain time stamped geographic positions. Given that an acoustic signal from a transmitter is detected by at least three hydrophones, the position of the transmitter at the time of signal emission can be calculated from the minute differences in time of arrival at each hydrophone using hyperbolic triangulation (for further technical details, see Cote et al. 1998 and Niezgoda et al. 2002). Moreover, as the data collection is automatic, unintentional disturbances of the study area can be limited to that associated with required hydrophone maintenance. Additionally, the number of study animals equipped with a transmitter can be chosen according to the sample size needed to answer a specific research question as it is not limited by a manageable observer effort.

By deploying an APT to cover an entire ecosystem, such as a small to medium sized lake, it is thus possible to turn a natural lake into a field-laboratory providing detailed behavioural information of tagged animals. This enables the testing of both novel and established research questions and theories under natural conditions. For instance, predator-prey interactions and interactions on multi-species and multi-trophic levels can be investigated under natural conditions in the field. Furthermore, by linking laboratory protocols and field observations, new insights in areas such as bioenergetics (Burton et al. 2011) and behavioural syndromes are conceivable (Sih et al. 2012). Additionally, behavioural responses to anthropogenic disturbances, e.g. angling and boating, and ecosystem manipulations, such as experimental introduction of invasive species and degradation or enhancement of habitat quality and quantity, can be studied on an individual level. Moreover, as the focal animals are studied within their natural habitat, genotype by environment interactions, which can be a significant source of experimental bias under laboratory conditions and potential lead to false conclusions (Brown et al. 2007; Klefoth et al. 2012), can be avoided.

To simultaneously and uniquely identify many tagged animals monitored by an APT system, the signals emitted from the transmitters need to be coded. This can be done using at least two markedly different methodologies. Conventional acoustic telemetry utilize pulse-position (PP) coding, in which information such as animal id and sensor data are embedded within the time intervals of a sequence of energy pulses. This method has a number of drawbacks including limitations in number of transmitters that can be monitored simultaneously in the same area (typically less than ten), poor code discrimination reliability under sub-optimal conditions and limitations in achievable accuracy. Alternatively, information from the transmitters can be encoded using code division multiple access (CDMA) technology, which is extensively used in e.g. cellular telephony and the Global Positioning System (GPS) (Niezgoda et al. 2002). This technology allows the simultaneous tracking of more transmitters with greater accuracy than the PP technology (see Niezgoda et al. 2002 for further discussion on PP and CDMA).

In recent years, a number of biological studies employing APT systems have been published, showing the potential of the APT technology (e.g. Cote et al. 2002; Zamora & Moreno-Amich 2002; Hanson et al. 2007; Hasler et al. 2009a; Svendsen et al. 2011; Rillahan et al. 2011; Baktoft et al. 2012). Whereas performance of a PP based APT system has been tested and reported (Espinoza et al. 2011), performance assessments of CDMA based APT systems have not yet been published. Therefore, the aim of the present study was to test the performance of two proprietary CDMA based APT systems deployed in two different lakes. Using a standardized protocol we assessed the efficiency, accuracy and precision of each system. Furthermore, as habitat structure and complexity are important factors in many ecological processes and can influence acoustic signal propagation (Diana et al. 1977), we tested the effect of habitat type and complexity on system performance.

Materials and Methods

Acoustic Positional Telemetry (APT) systems

The study was conducted in two lakes each equipped with an APT system based on the CDMA technology (Niezgoda et al. 2002) operating at 200 kHz frequency. Both systems were deployed to cover the entire respective lake. Lake1 (Kleiner Döllnsee, 52°59´ N, 12°34´ E, Germany, 24 ha) was equipped with a wireless system (WHS 3050; Lotek Wireless Inc, Newmarket, Ontario, Canada) whereas Lake2 (Lake Gosmer, 55°55´ N, 10°10´ E, Denmark, 1 ha) was equipped with a cabled system (MAP600; Lotek Wireless Inc, Newmarket, Ontario, Canada). The ALPS in Lake1 consisted of 20 wireless hydrophones with integrated dataloggers, synchronized using dedicated beacon transmitters attached to each hydrophone. The APT in Lake2 consisted of eight cabled hydrophones connected to a single onshore datalogger. While the hardware setup in the two lakes was dissimilar, both systems utilized comparable technology and the same rationale for calculating positions of the transmitters. In short, coded signals emitted from transmitters were detected by hydrophones and stored by a logger. The stored data was downloaded to a portable computer for subsequent analysis using proprietary software (Lake1: ALPS, ver. 2.22; Lake2: BioMAP ver. 2.1.12.1; both from Lotek Wireless Inc). If an emitted signal was detected by at least three hydrophones, a 2D position was calculated based on the minute differences in time of arrival at each hydrophone using hyperbolic triangulation techniques (for further information, see Cote et al. 1998; Niezgoda et al. 2002). The transmitters used in Lake1 was CH-TP16 (burst interval 9.2 s, but every fourth signal was relayed as a radio signal and thus not detectable by the APT; Lotek Wireless Inc) and in Lake2 MAP6_2 (burst interval 2.56 s; Lotek Wireless Inc) in Lake2. Although being different models, the used transmitters were technically comparable and used similar frequency and coding. Besides identification code the transmitters used in Lake1 relayed information from an integrated pressure sensor enabling calculation of the depth of the transmitter.

Data processing and filtering

The calculated positions originating from the proprietary software contain a variable amount of system induced outliers (Niezgoda et al. 2002). To remove extreme cases of these a slight filtering based on proprietary metrics (Dilution Of Precision (DOP), Condition Number (CN) and Reliability Number (RN)) was applied (the metrics are described Niezgoda et al. 2002) using the following values: DOP < 10, CN < 10, RN > 0. The resulting data are henceforth referred to as ‘raw’ positions. To further and objectively reduce the effect of system induced spurious outliers in the raw data, the raw positions were filtered using stricter values (DOP < 1, CN < 10, RN > 1) and subsequently smoothed using a Hidden Markov Model based approach with a t-distributed observation noise (Pedersen et al. 2008; Pedersen et al. 2011) yielding the most probable track of each transmitter, henceforth referred to as 'filtered' positions.

Due to limitations in the proprietary software, position calculations could only be based on data from a maximum of eight hydrophones at a time. Thus, to fully utilize the data from all 20 hydrophones in Lake1 several arrays consisting of eight hydrophones each were defined and processed separately. However, as the signals often were detected by several hydrophone arrays, this resulted in multiple calculated positions originating from the same signal. Furthermore, as the individual hydrophone arrays were not completely in sync with each other, the timestamps of the multiple calculated positions from the same signal differed slightly. Thus, the raw positions from Lake1 were processed to ensure that the multiple calculated positions from each emitted signal had identical timestamps prior to applying the Markov filter. The data produced by the Markov filter contains only one position per timestamp representing the most probable position based on all available information.

Procedures to assess system efficiency, accuracy and precision

To assess efficiency, accuracy and precision of the two systems a number of stationary tests were conducted (Lake1: n = 155; Lake2: n = 123). During the tests, transmitters were moored at known positions (± 0.2 meter using a differential gps (DGPS) unit, Trimble GeoXH, Sunnyvale, California, USA) for a period of time allowing the transmitters to emit several hundred signals. The transmitters were deployed to mimic potential positions of fish, by attaching them to a line, held in place by a heavy sink and kept vertical by a float. Up to four transmitters were attached to each line at known distances from the water surface. Each deployment of a transmitter is treated as a sampling unit in subsequent analyses. The habitat structure and complexity at the sites of deployment was categorized based on depth and the type and quantity of the vegetation in immediate vicinity of the transmitter. The following habitat categories were used: dense emergent macrophytes (ED), loose emergent macrphytes (EL), dense submerged macrophytes (SD), above submerged macrophytes (SA), shallow open water (OS) and deep open water (OD). All six habitat categories were present in Lake1 whereas Lake2 did not contain sufficient amounts of submerged macrophytes to facilitate transmitter deployments in these, i.e. categories SA and SD are not included in data from Lake2.

System efficiency was defined as the proportion of emitted signals resulting in a calculated position. Accuracy of the calculated positions was defined as the deviation from the true position, i.e. the Euclidian distance between calculated and true position. For each stationary test, accuracy was calculated as the mean of these distances. Precision was defined as the variability of those distances and calculated for each stationary test as the standard deviation of the overall accuracy. Efficiency, accuracy and precision were calculated for both raw and filtered positions.

For each trial in Lake1, the distance from transmitter to the water surface, henceforth referred to as true transmitter depth, was measured, thereby allowing an assessment of the depth reported by the transmitters.

Tow test

The stationary tests were obviously inadequate to assess system performance for moving animals. Therefore, a number tow tests (Lake1: n = 6; Lake2: n = 22) were conducted in each lake to mimic trajectories of fish swimming around and across the lake following a linear to curvilinear path. During these, three transmitters were attached to a solid vertical rod mounted on a boat, thus yielding 18 and 66 trials. The true trajectory was determined using a DGPS positioned directly above the transmitters. System efficiency for moving transmitters was assessed following the protocol from the stationary tests whereas accuracy calculations were based on minimum distances between calculated positions and the DGPS trajectory. Precision could not be calculated for the tow tests as the transmitters were moving.

Statistics

Potential effects of habitat type and complexity on efficiency, accuracy and precision on the filtered data were analysed by fitting linear models using generalized least squares (gls; Zuur et al. 2009), thereby allowing for unbalanced data sets. In each test, habitat type was entered as fixed factor and trial means of efficiency, accuracy or precision as dependent variable. Thus, a total of six models were fitted, one for each of the dependent variables in each lake. Habitat categories with less than four trials yielding positions were excluded from the analysis. Prior to fitting the models, efficiency was arcsin-transformed and both accuracy and precision was log(y + 0.1) transformed, according to Zar (1999). A variance structure was included to allow for heterogeneity of variances between habitat categories when this significantly improved model fit (Zuur et al. 2009). The relationship between true transmitter depth and depth reported by the transmitters were analysed by fitting a linear model using a general linear model (GLM). Trial means of reported depth was entered as explanatory variable and true transmitter depth as dependent variable. Habitat type was not included in this analysis as the transmitter depth measurement is based on pressure sensor technology and therefore not influenced by habitat type. Statistical analysis were done in R version 2.10.1 (R Development Core Team 2010) using the nlme 3.1-97 package (Pinheiro et al. 2010) in addition to core functions.

Results

Stationary tests

There was a significant effect of habitat category on efficiency, accuracy and precision in both lakes (gls; $p < 0.01$ in all tests; Table 1; Fig. 1). For all three performance measures, the non-structured open water habitats yielded best performance, whereas structured habitats generally reduced performance dependent on macrophyte density (Fig. 1; Table 1). Additionally, there was considerable variation within habitat category in all performance measures in both lakes (Fig. 1; Table 1), indicating that other factors than habitat category influence system performance.

The application of the Markov filter improved both accuracy and precision substantially compared to the raw data, although the final filtering step resulted in a slight decrease in system efficiency (Fig. 1).

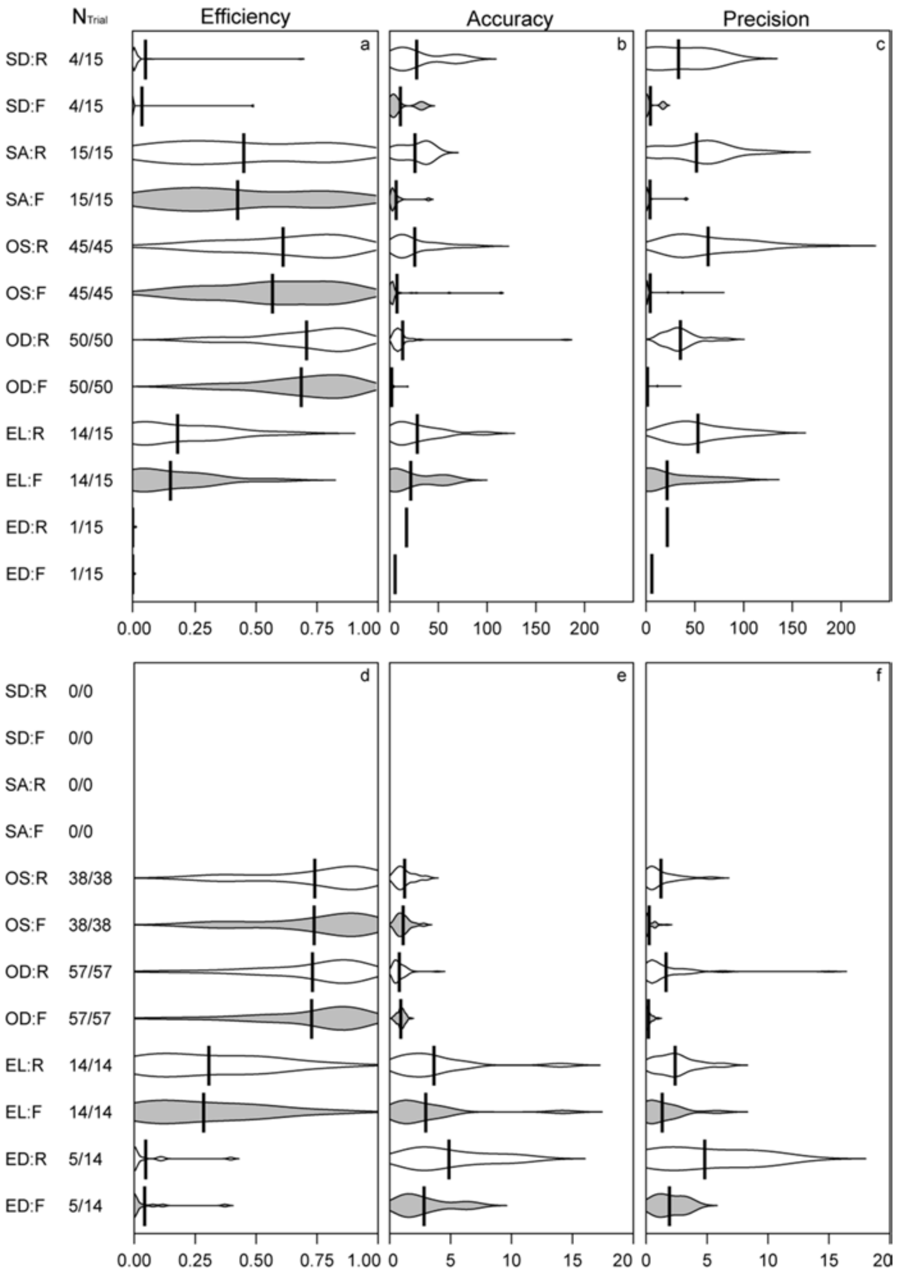

Fig. 1. Distributions of mean efficiency (a, d), accuracy (b, e) and precision (c, f) from Lake1 (a, b, c) and Lake2 (d, e, f) in the six different habitat types (SD = dense submerged macrophytes; SA = above submerged macrophytes; OS = shallow open water; OD = deep open water; EL = loose emergent macrophytes; ED = dense emergent macrophytes). R and F denote raw (white) and filtered (grey) data, respectively. Height of the plots indicates data point density. Mean values are given by solid vertical bars. N_{Trial} indicates number of trials yielding positions / number of trials in each habitat type. The x-axes in panels a and c are dimensionless and meters in panels b, c, e and f. Note different scales on the x-axes

Table 1. Coefficient estimates (back transformed) from the linear models. Numbers in brackets are the coefficient estimates minus and plus standard deviation which is non-symmetrical due to the back-transformation.

Lake1

Habitat type	Efficiency (%)		Accuracy (m)		Precision (m)	
SubmDense (SD)	0.7	(0.1 - 3.5)	6.1	(1.8 - 20.6)	0.8	(0.0 - 6.8)
SubmAbove (SA)	41.6	(12.5 - 74.5)	4.4	(2.0 – 10.0)	1.5	(0.4 - 4.8)
OpenShallow (OS)	58.1	(31.2 - 82.7)	3.1	(1.1 - 9.1)	1.1	(0.2 - 3.9)
OpenDeep (AD)	70.8	(49.3 - 88.4)	1.9	(1.2 – 3.0)	0.6	(0.2 - 1.4)
EmerLoose (EL)	11.6	(1.2 - 30.8)	10.3	(2.6 - 40.3)	3.9	(0.3 - 39.2)
EmerDense (ED)	0.0	(0 - 0)	-	-	-	-

Lake2

Habitat type	Efficiency (%)		Accuracy (m)		Precision (m)	
SubmDense (SD)	-	-	-	-	-	-
SubmAbove (SA)	-	-	-	-	-	-
OpenShallow (OS)	76.5	(52.2 - 94.0)	1.0	(0.6 - 1.7)	0.1	(0.0 - 0.5)
OpenDeep (AD)	74.7	(50.0 - 92.9)	0.9	(0.6 - 1.3)	0.1	(0.0 - 0.4)
EmerLoose (EL)	24.7	(6.7 - 49.2)	1.8	(0.6 - 5.2)	0.6	(0.1 - 2.7)
EmerDense (ED)	1.2	(0.2 - 6.9)	2.3	(1.1 - 4.7)	1.6	(0.7 - 3.4)

Depth

There was a strong linear relationship between true transmitter depth and depth reported by the transmitters (Fig. 2; n = 155, GLM, no intercept, coefficient estimate = 1.01, std. error = $7.5 * 10^{-3}$, $p < 0.0001$). Furthermore, the within-trial variation was small (mean standard deviation = 0.06 m). These findings indicate that the depths reported by the transmitters were correct and consistent.

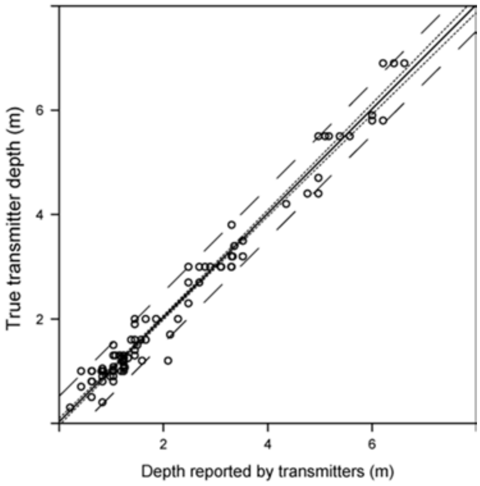

Fig. 2. Depth measured in the field versus depth reported by the stationary transmitters. Solid line is the regression line, broken and dotted lines are 95 % confidence and predictions intervals, respectively (n = 155, GLM, no intercept, coefficient estimate = 1.01, std. error = $7.5 * 10^{-3}$, $p < 0.0001$).

Tow tests

Mean trial efficiency of the tow tests was 0.51 (SD = 0.18) and 0.86 (SD = 0.11) in Lake1 and Lake2, respectively. Corresponding accuracies was 5.27 m (SD = 4.0) and 0.43 m (SD = 0.16) (Fig. 3). Mean speed of the boat during the tow tests was 0.58 m/s (SD = 0.16) in Lake1 and 0.43 m/s (SD = 0.066) in Lake2.

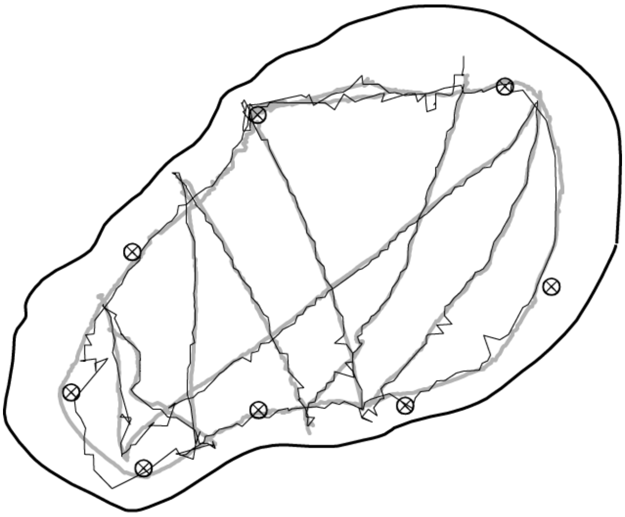

Fig. 3. Visualisation of a subsample of the tow tests conducted in Lake2. Tracks of nine tows are shown. Only the track from a single of the three used transmitters is shown. Thick black line indicates perimeter of the lake. Overall there was good concordance between calculated (thin, black line) and true trajectory (grey line). However, accuracy can decline when the transmitter is outside the footprint of the hydrophone array as evident in the south-west corner.

Discussion

The present study assessed the performance of two APT systems deployed in two freshwater lakes of different sizes by studying the efficiency, accuracy and precision of the systems. In both lakes, system performance was dependent on habitat category with decreased performance in structural complex habitats. Furthermore, within habitat category variation in the performance parameters was relatively large. The findings reflect the complex nature of aquatic acoustic positional telemetry. In general, the probability of detecting a signal at a given hydrophone is a function of the amount of signal attenuation caused by the water itself and obstacles such as macrophytes, and signal interference from multipath propagation caused by signal reflection from hard substrates or the water surface (MacLennan & Simmonds 1992). Furthermore, an emitted signal needs to be detected by at least three hydrophones to facilitate a position calculation. Thus, as signal attenuation *ceteris paribus* is positively correlated with habitat complexity, the observed decrease in system efficiency in complex habitats was expected. The within and between habitat category variation in both accuracy and precision can likely be explained by the position-dependent variability inherent in the hyperbolic triangulation algorithm (Niezgoda et al. 2002). Generally, the performance of the algorithm peaks at positions within the centre area of an equilateral triangle outlined by three hydrophones and degrades gradually for positions closer to and outside the triangle edges. Thus, positions outside the hydrophone array will generally be less accurate than positions inside (Niezgoda et al. 2002; Zamora & Moreno-Amich 2002). Alternatively, it is conceivable that complex habitats can cause more signal interference than non-structured open water habitats and, therefore, the habitat category *per se* can influence system accuracy and precision. However, in the present study the effect of hydrophone geometry on system accuracy and precision could not be separated from the potential effect of habitat category as none of the structured habitat categories were present both within and outside the hydrophone arrays.

The APT in Lake2 generally performed better than the APT in Lake1. This can most likely be explained by differences in lake size and hydrophone array configuration. Lake1 was approximately 20 times larger than Lake2 and, although more hydrophones were deployed in Lake1 to accommodate limits in acoustic range and signal blocking caused by lake bathymetry, the coverage of Lake1 in terms of between-hydrophone distances and area outside the hydrophone array was coarser. Furthermore, the proprietary positioning algorithm could only utilize a maximum of eight hydrophones simultaneously necessitating that multiple arrays, of up to eight hydrophones each, needed to be defined. In effect, this divided the whole-lake coverage of the twenty hydrophones into several smaller arrays, each covering a relatively small proportion of the entire lake. Therefore, a relatively large number of the stationary tests resulted in calculated positions that were well within the coverage of the twenty hydrophones, but were outside the array that actually calculated the position. Ultimately, this might have led to lower accuracy and precision.

The calculated positions produced by the proprietary software were encumbered by a relatively large number of spurious outliers, resulting in poor accuracy and precision of the raw data in both lakes (see Fig. 1). However, following the application of a Hidden Markov model (Pedersen et al. 2008; Pedersen et al. 2011) system performance improved substantially. The Hidden Markov approach was chosen as it processes the data by estimating “the most probable track” of the transmitters by analysing each individual position while considering the preceding and following positions.

The presence of spurious outliers was especially true for Lake1, in which the reduced quality of the raw data was partly caused by the limitations in the proprietary positioning software (limit of eight hydrophones). Although this limitation can be remedied by applying a multi-array structure as done in Lake1, researchers should be aware of potential challenges derived from this approach. These include multiple calculated positions originating from a single emitted signal caused by the signal being detected by more than one array. As discussed above, this will lead to a relatively large number of position calculations based on poor hydrophone geometry and, thus, poor accuracy and precision. Time drift of the internal clocks in the wireless hydrophones causing de-synchronization of the defined arrays will further complicate this, as it leads to different time stamps on the multiple positions originating from a single signal.

Previous studies utilizing CDMA based APT systems often claim ‘sub-meter accuracy’ of their systems; either with reference to Niezgoda et al. (2002) or Cooke et al. (2005) (Hanson et al. 2007; Hasler et al.

2009b; O'Connor et al. 2010), own observations (Cote et al. 1998) or unreferenced (Cote et al. 2002; Cote et al. 2003). Although, the papers referred to (Niezgoda et al. 2002; Cooke et al. 2005) both mention that sub-meter accuracy is achievable, they do not provide rigorous standardized tests of whether this claims is general, and thus valid, for the entire extent of their study site. The results from the present study support the claim that sub-meter accuracy (and precision) is indeed possible. However, the findings also reveal that, at least in the two systems studied, this is not unambiguously correct.

The tow tests revealed that the APT systems performed equally well for stationary and moving transmitters as efficiency and accuracy were similar to the stationary tests. Furthermore, the results illustrate the effect of hydrophone geometry on position accuracy, e.g. the degradation of accuracy as the transmitters are moved outside the hydrophone array (see Fig. 3, south-west corner). It should be noted that the accuracy estimates of the tow tests potentially are downward biased since the error of each calculated position on the axis parallel to the DGPS trajectory was ignored. However, as the true positions of the transmitters at the exact times of signal emissions were unknown, this component of the total positional error was unobtainable. Nevertheless, the tow tests verified that the APT systems were capable of tracking the moving transmitters, which, of course, is a crucial requirement for the applicability of the systems to successfully study free ranging animals.

In summary, we conclude that both the deployed APT systems were capable of providing positional data of a high quality in terms of both temporal and spatial resolution. We advocate that, although sub-meter accuracy is not to be expected throughout a study site, the CDMA based APT systems are indeed a potent tool for studying aquatic animal behaviour and offer a major improvement to traditional telemetry methods regarding the combined temporal and spatial resolution of positional data.

Comments and recommendations

The effects of habitat complexity and hydrophone geometry on system performance should be considered during the design phase of studies employing APT systems. For instance, focal species that are known to reside in complex habitats close to the shore would warrant a different hydrophone array configuration than pelagic species primarily residing in the centre of a lake in order to facilitate better coverage. Moreover, as each lake is unique in terms of bathymetry and acoustic properties, we strongly advocate that rigorous tests of system performance should be considered an integral part of the initial phases of studies using APT systems. Without a proper knowledge on the performance of a particular system setup, erroneous interpretations of obtained data and, subsequently, false conclusions are prone to be made. Because the systems performances in our study could be significantly improved by application of a Hidden Markov Model, researchers are advised to consider the possibility of using such statistical options if high spatial resolutions are needed for answering the research questions. Additionally, we advise researchers to ensure that they have access to the knowhow required to process raw data as well as data handling and analyses of the potentially very large data sets obtained using APT systems. This includes the development of databases and analysis algorithms, which, to a large extent, will need to be custom designed for each specific research project.

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Seasonal and diel effects on the activity of northern pike studied by high-resolution positional telemetry

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Seasonal and diel effects on the activity of northern pike studied by high-resolution positional telemetry

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Abstract – Temperate lakes can be ice covered for several months each year, yet little is known about the behaviour and activity of the fish during the cold season. As northern pike represents the top of the food web in many northern temperate lakes and may structure the ecosystem both directly and indirectly, a detailed understanding of the behaviour of this species during winter is important. We continuously monitored the activity of adult northern pike (*Esox lucius*) in a small temperate lake from late summer to winter for two consecutive years using an automatic acoustic positional telemetry system. Four subsample periods representing different temperature regimes from each year were chosen for further investigation. The results revealed that pike activity was similar between seasons. In all periods, a distinct diel pattern, showing increased activity during day as compared to night, was evident. Our findings indicate that the fish component of temperate lentic ecosystems can be more active during the cold season than previously assumed. This may have implications for the structuring effect of pike on the lower trophic levels.

Key words: winter biology; temperate lake; activity; Esox; diel patterns

Introduction

The winter season in temperate lakes is often considered to be a period of very low activity and production and thus an ecologically insignificant period (Salonen et al. 2009). The notion of an inactive ecosystem, combined with the logistic and methodological challenges involved in winter field studies, has implicated that the general knowledge on lake winter ecology is limited (Salonen et al. 2009). However, evidence is mounting that lake winter ecology is not static, at least regarding the activity and behaviour of the fish (e.g., Jurvelius & Marjomaki 2008; Amundsen & Knudsen 2009; Salonen et al. 2009).

In many subarctic and temperate lakes in the Northern Hemisphere, northern pike (*Esox lucius*), henceforth termed pike, is the natural apex predator (Craig 1996). Besides a regulatory effect on prey fish abundance and population dynamics (Byström et al. 2007), more subtle nonconsumptive predator effects of pike on prey fish communities are known, including changes in morphology (Brönmark & Miner 1992; Eklöv & Jonsson 2007), reduced activity (Lima & Dill 1990; Bean & Winfield 1995; Jacobsen & Berg 1998) and altered habitat utilisation (Jacobsen & Perrow 1998). By altering prey fish abundance and behaviour, pike can affect the local ecosystem, including nutrient cycles and levels, through cascading top-down effects (Prejs et al. 1994; Berg et al. 1997; Skov et al. 2011) underpinning the ecological importance of this species. Although the mechanisms mediating prey fish response are variable and complex, both visual and chemical cues from pike have been found to alter prey fish behaviour (Mikheev et al. 2006; Martin et al. 2010). Pike are visual predators (Casselman 1996) whose activity typically follows a diel pattern showing

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lowest activity at night and higher activity during the day, and activity peaks at dusk and dawn (Diana 1980; Cook & Bergersen 1988; Beaumont et al. 2005). However, given the ecological importance of this species, relatively little is known about the detailed pike winter biology including activity level and behaviour.

In ectothermic animals, the upper limit of energy available to, for example, behavioural activity is primarily controlled by the temperature of the surrounding media through the temperature dependency of metabolic rates and enzymatic activity (Fry 1971). Therefore, it is conceivable that volitional activity could be positively correlated with water temperature. This correlation has indeed been found in a laboratory study, showing that pike swimming activity peaks at approximately 20 °C and gradually decreases to a very low level at 6 °C (Casselman 1978). Corroborating this, Cook & Bergersen (1988) and Kobler et al. (2008) found reduced swimming activity during winter as compared to summer. Contrasting these findings, other studies found winter activity to be similar or even higher than summer activity (Diana et al. 1977; Jepsen et al. 2001; Koed et al. 2006). These discrepancies might reflect biological variability in pike behaviour in different ecosystems. However, the methodologies implemented are diverse, potentially invalidating direct comparisons between studies. Furthermore, the above-mentioned field studies used telemetry with manual tracking protocols that can have repercussions on the quality of the acquired data. For instance, a trade-off between temporal resolution and the number of fish tracked is inevitable. Moreover, the manual tracking process *per se* may disturb the tagged fish and thereby induce nonvolitional behaviour biasing the data. A recently developed alternative to manual tracking is acoustic positional telemetry systems (APTS), enabling continuous automatic positional telemetry (Niezgoda et al. 2002). This method effectively surmounts potential imperfections of manual tracking. A large number of fish can be positioned simultaneously with high temporal and spatial resolution and, as the data collection is carried out automatically by stationary hydrophones, the risk of disturbing the focal fish as part of the tracking process is eliminated.

In this study, we aimed at increasing the knowledge on winter biology of pike. Specifically, patterns and levels of activity, in terms of movement, are investigated and compared between seasons. By implementing an APTS, a very detailed data set on pike behaviour in a natural environment was acquired. Pike were continuously positioned through two consecutive years. From this data set, subsamples from late summer through winter in both years were selected for further analysis.

Materials and methods

Study area

The study was conducted in a small Danish eutrophic temperate lake [Lake Gosmer; 55°9′N, 10°2′W; area approximately 1 ha; ellipse-shaped, ca. 140 × 80 m; maximum depth 8 m; mean annual Secchi depth 1.2 m (range: 0.4–2.4 m)]. The lake is an isolated water body, that is, there are no connected streams. The fish community was dominated by pike, perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*). Submerged vegetation was restricted to a single bed of water lilies (*Nuphar lutea*) along the southern shore. Narrow and dense stands of emergent macrophytes (*Typha latifolia*) covered the majority of the shoreline apart from the northern shore that was shaded by overhanging trees.

Environmental variables

Water temperature and dissolved oxygen content were registered at four depths through the water column (1.0 m, 2.5 m, 4.0 m and 5.5 m from the surface) by stationary data loggers (FDO 700 IQ probes, IQ Sensor Net, WTW GmbH, Weilheim, Germany). Calculations of mean water temperature and oxygen content were based on data from the loggers at 1.0 and 2.5 m. Lake volumes were calculated using GIS software (ArcGis 9.3, ESRI, Redlands, CA, USA).

Fish

During the period March 2009–October 2010, a total of 29 pike were captured using rod and line, trammel nets or electro fishing on several fishing occasions and tagged at the time of capture. Pike were anesthetised in 0.5 $mg \cdot l^{-1}$ solution of 2-phenoxyethanol, and an acoustic transmitter (Lotek MA-TP11-25; 11 mm diameter; 61 mm long; 11 g in air; 45 s burst interval, Lotek Wireless Inc., Newmarket, Ontario, Canada) was inserted into the body cavity through an incision anterior to the pelvic fins. The incision was closed by two separate sutures using absorbable suture material (Vicryl 3-0 FS-2, ETHICON, Johnson & Johnson Medical Limited, Livingston, Scotland). Total length and body mass were registered. Duration of the complete tagging procedure was 3–5 min. Finally, pike were released following full recovery in aerated lake water. A previous study using a comparable tagging procedure (Jepsen & Aarestrup 1999) found no or only minor adverse effects on growth and survival of pike.

Tracking system

The study lake was equipped with an APTS (Lotek MAP_600; Lotek Wireless Inc., Newmarket, Ontario, Canada) that continuously monitored the tagged fish. In short, a receiver stored data collected by eight hydrophones covering the entire lake. The data were processed using proprietary software (BioMap v. 2.1.12.1; build 2.633; Lotek Wireless Inc., Newmarket, Ontario, Canada) yielding raw calculated positions based on registration by at least three hydrophones (Niezgoda et al. 2002). The raw positions were filtered and smoothed using a hidden Markov model–based approach with a t-distributed observation noise (Pedersen et al. 2008). Validity of the tracking system was verified by monitoring transmitters moored at known positions and transmitters towed by a boat (Baktoft, unpublished results).

Data analysis and statistics

From the complete data set, four subsample periods (PER) through late summer, autumn and winter were selected for further analysis for two consecutive years, 2009 and 2010. Each period consisted of five consecutive days. The periods were selected to represent different seasons and thus water temperature regimes while keeping other environmental factors as identical as possible. The lake was left undisturbed, for example, no boat traffic, sampling or fishing while data were collected.

Distance calculations

Activity of each individual pike was calculated as the minimum moved distance per hour (DPH_{ind}) based on observations within 60-min periods. Thus, in each of the eight PERs, a total of 24 × 5 = 120 measures of DPH_{ind} were made on each individual pike included in the respective period. DPH_{ind} was defined as the two-dimensional Euclidian distance between consecutive locations. As the transmitters used had a burst interval of 45 s, each individual was positioned up to 80 times every hour, yielding a maximum of 79 calculated distances per hour. However, the actual number of positions per hour (N_{obs}) varied between zero and full coverage (79 calculated distances) because of the probabilistic nature of detecting the acoustic signals.

As observed activity is estimated as the sum of line segment lengths, the maximum obtainable observed activity (DPH_{ind}) will inevitably be a function of N_{obs}. However, as the true level of activity is a finite value, there exists a given threshold of N_{obs} above which the observed activity credibly reflect true activity. Visual inspection of mean DPH_{ind} plotted against N_{obs} revealed that at $N_{obs} < 24$, there was a positive correlation between N_{obs} and DPH_{ind}, indicating that DPH_{ind} was underestimated. At $N_{obs} \geq 24$, this correlation was absent. Therefore, all data with $N_{obs} < 24$ were excluded from further analysis, reducing the data set from 19,440 to 10,606 observations on DPH_{ind}.

Mean moved distance for each hour of the day ($DPH_{ind\ mean}$) within each PER:Year combination was calculated as the mean of DPH_{ind} means grouped by time of day (TOD; [0:23] each representing an hour). Thus, for each TOD in each PER:Year combination, the mean moved distance was calculated for each individual pike. Subsequently, the mean and variance of these individual means were calculated to get the overall mean moved distance ($DPH_{TOD\ mean}$) and corresponding variance for each TOD grouped by PER:Year. Finally, these were summed within each PER:Year combination to get mean daily moved distances. Variances of the $DPH_{TOD\ mean}$ values were summed and square rooted to give standard deviations (SD) of the daily means.

Statistics

Statistical analyses were performed to investigate how overall activity and diel patterns of activity varied between periods and years. Initial analysis according to Zuur et al. (2009) revealed (i) no extreme outliers present in the data set, (ii) potential interactions between PER:TOD and PER:Year and (iii) the effect of TOD on DPH_{ind} was complex and nonlinear. DPH_{ind} was log10(y + 1)-transformed to meet the assumption of normality. Data were analysed using a mixed-effects model with random intercept including individual fish (FishID) as a random factor. This model imposes a compound symmetry correlation structure allowing multiple observations from the same individual to be correlated (Pinheiro & Bates 2000). Furthermore, a first-order autoregressive correlation structure (AR1; Pinheiro & Bates 2000) was included to model the temporal correlation between observations within each FishID nested in PER and Year. The model was further extended to a generalised additive mixed-effects model (GAMM; Wood 2006), thereby allowing the nonlinear TOD-DPH_{ind} relationship to be fitted by smoothers. Cyclic cubic regression splines were used to model the cyclic nature of TOD (Wood 2006). Finally, total length, centred around 60 cm (TL60), was included as a covariate. By centring total length, the estimated intercept from the GAMM represents a 60-cm pike instead of a 0-cm pike.

In summary, the following GAMM was fitted:

$$\begin{aligned}\mathrm{Log}_{10}(\mathrm{DPH}_{\mathrm{ind}}+1) = {} & \alpha + \mathrm{TL60} + \mathrm{PER} + \mathrm{Year} \\ & + \mathrm{PER{:}Year} \\ & + f(\mathrm{TOD}){:}\mathrm{PER{:}Year} \\ & + a + \varepsilon\end{aligned}$$

where α is the overall intercept; TL60 is total length centred around 60 cm; PER is period of year; f(TOD) is a smoothing function modelling time of day using a cyclic cubic regression spline; a is a random component that is normally distributed with expectation 0 and variance σ_a^2; and, finally, ε is the error term, which is modelled by the AR1-structure along with random noise following a normal distribution. The effect on model fit of all included terms and interactions was tested by comparing full and nested models using Akaike information criterion (AIC) as described by Zuur et al. (2009) and Wood (2006). In short, AIC is a measure of goodness of fit that incorporates model complexity; the lower the AIC, the better the model fits the data. Terms and interactions that did not improve model fit were removed.

Statistical analyses were performed in R version 2.12.1 (R Development Core Team 2010) using the mgcv 1.7-2 (Wood 2006) and the nlme 3.1-97 (Pinheiro et al. 2010) packages.

Results

The number of pike and summary of environmental variables in each period are given in Table 1. A thermocline at approximately 4 m from the surface was present during PER1 in both years, rendering the hypolimnion anoxic. The hypolimnion constituted approximately 17% of total lake volume. No stratification was present in other periods. The number of pike varied, because of the longitudinal nature of the study and associated natural mortality of tagged and sequential tagging of new pike.

Mean daily movement ranged from 621 to 938 m·day^{-1} in 2009 and 928 to 1248 m·day^{-1} in 2010. Although between-period variation was apparent, there was no consistent seasonal trend in mean daily movement (Fig. 1).

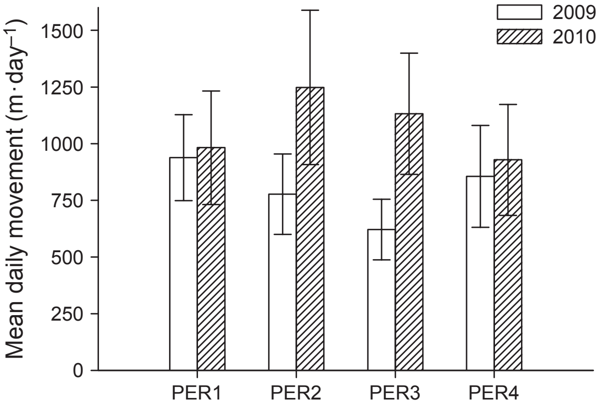

Fig. 1. Observed mean activity per day (m·day^{-1}) in the four periods (PER) grouped by year (2009: open bars; 2010: hatched bars). Error bars indicate standard deviation (SD). Mean values are calculated as the sum of the means of individual means per time of day (TOD).

In general, a distinct diel pattern was evident showing lowest activity at night, activity peaks around sunrise and sunset and intermediate activity during the day (Figs 2 and 3). Timing of the twilight-related peaks followed the seasonal changes in time of sunrise and sunset. This pattern did show some variability, especially in 2010 where activity during the night remained relatively high in periods one and two. However, although the diel patterns were variable, the applied GAMM revealed that they were best modelled by one smoother for each period representing both years (Table 2, Fig. 3). This indicates that, generally, the diel patterns varied between the periods, and that the diel pattern in each period was similar between years. However, overall activity levels in each period varied between years, as indicated by the PER:Year interaction, with the fish generally being more active in 2010 (Table 2, Fig. 1).

Table 1. Characteristics of the periods (PER) included in the study. Num pike gives the number of tagged pike in each period; TL gives mean total length of the tagged pike in centimetres, minimum and maximum are given in parentheses; temp and O_2 give mean water temperature and mean oxygen content as well as standard deviations.

		Start date	Num pike	TL (cm) (min–max)	Temp (°C) (SD)	O_2 (mg l^{-1}) (SD)
2009	PER1	August 30, 2009	11	68 (46–99)	17.3 (0.4)	6.4 (1.2)
	PER2	October 15, 2009	21	65 (42–99)	8.4 (0.3)	6.8 (0.6)
	PER3	December 9, 2009	20	64 (36–99)	5.0 (0.3)	8.9 (0.1)
	PER4*	January 7, 2010	20	64 (36–99)	1.9 (0.0)	6.7 (0.9)
2010	PER1	September 9, 2010	21	65 (49–99)	15.5 (0.2)	5.7 (0.9)
	PER2	October 20, 2010	23	64 (49–99)	8.4 (0.4)	6.3 (0.4)
	PER3	November 18, 2010	23	64 (49–99)	4.5 (0.2)	9.2 (0.4)
	PER4*	December 23, 2010	23	64 (49–99)	1.0 (0.0)	7.8 (0.8)

*Indicates periods with at least 15 cm of ice covering the lake.

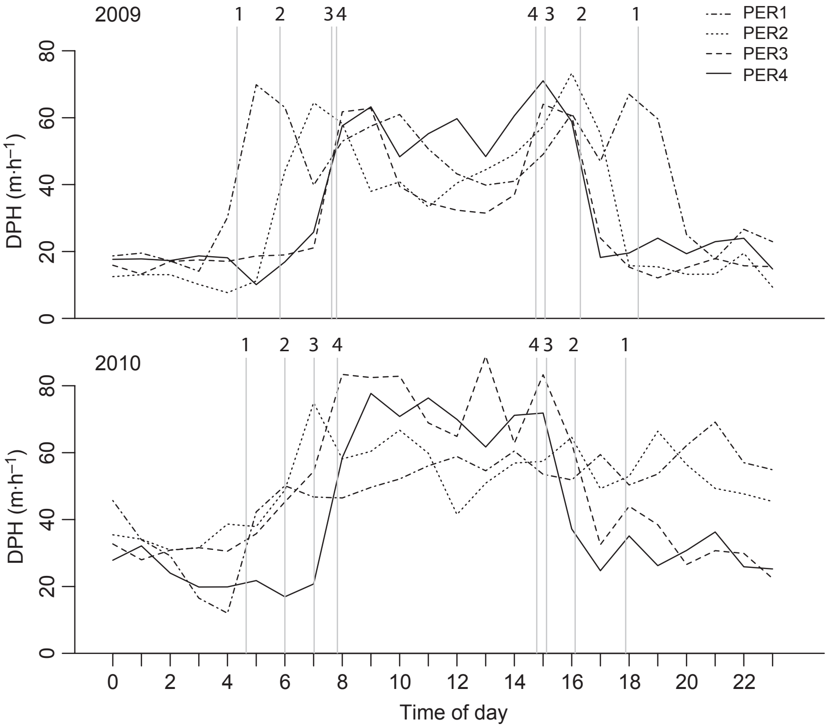

Fig. 2. Observed mean moved distance per hour ($DPH_{TOD\ mean}$) in 2009 and 2010 as a function of time of day (TOD) grouped by period (PER). Times of sunrise and sunset in the four periods are given by the vertical lines (corresponding period indicated by numbers above lines).

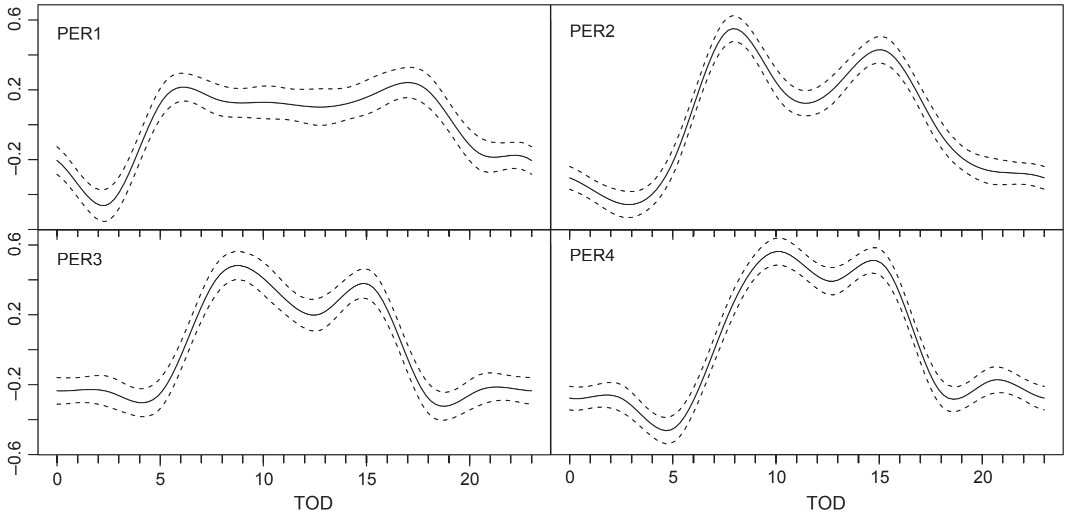

Fig. 3. Generalised additive mixed-effects model smoothing curves representing the modelled nonlinear effect of time of day (TOD) on activity. These plots illustrate the pattern of the TOD effect within each period. Note that the actual values of the TOD effect is further influenced by the other fixed effect terms including PER, that is, the PER main effect and the PER:Year interaction. Thus, the shape of the TOD effect, for example, timing of the peaks and difference between night and day, can be directly compared between PER, but not the activity values *per se*. Best fit of the model was achieved using a separate smoothing function for each period, thereby modelling an interaction between TOD and period (PER). Dashed lines represent 95% confidence intervals around the main effects.

Model validation by graphical inspection of the residuals indicated homogeneity and normality of the residuals. The AR1 correlation structure effectively modelled the temporal autocorrelation as judged by autocorrelation plot of the residuals.

Table 2. Numeric results from the fixed component of the GAMM; see Fig. 3 for smoothing curves of the TOD:PER interaction. (a) The optimal model was determined using Akaike information criterion (AIC) to exclude fixed effect terms and interactions that did not improve model fit. Models excluding main effects of PER, Year and TOD were not tested as these effects were included in interactions. (b) Fixed effect parameters estimated using restricted maximum likelihood (REML). Note that the intercept represents the expected activity of a 60 cm pike because of the centring of TL.

	(a) Model selection using AIC		
Model	d.f.	AIC	ΔAIC_{Final}
1 Full model[1]	20	14 423	50
2 Final model[2]	16	14 373	–
2a – TL60	15	14 380	7
2b – PER:Year	13	14 395	22
2c – TOD:PER	13	14 631	258

	(b) Fixed effect parameter estimates (REML)			
	Estimate	SE	*t*-value	Pr(>ltl)
Intercept	1.202	0.054	22.437	<0.0001
TL60	0.009	0.003	3.072	0.002
PER1	0	–	–	–
PER2	−0.211	0.046	−4.566	<0.0001
PER3	−0.209	0.049	−4.281	<0.0001
PER4	−0.228	0.047	−4.850	<0.0001
Year2009	0	–	–	–
Year2010	−0.007	0.049	−0.135	0.892
PER1:Year2010	0	–	–	–
PER2:Year2010	0.254	0.062	4.113	<0.0001
PER3:Year2010	0.240	0.066	3.655	<0.001
PER4:Year2010	0.062	0.062	1.002	0.317

[1] Full model: $\text{Log}_{10}(\text{DPH}_{ind} + 1) = \alpha + \text{TL60} + \text{PER} + \text{Year} + \text{PER:Year} + f(\text{TOD})\text{:PER:Year}$

[2] Final model: $\text{Log}_{10}(\text{DPH}_{ind} + 1) = \alpha + \text{TL60} + \text{PER} + \text{Year} + \text{PER:Year} + f(\text{TOD})\text{:PER}$

Discussion

Daily moved distance remained at a relatively constant level through the seasons in both years and, although between-year variation was apparent, the pike were active in all periods. There was no consistent seasonal trend in the activity of the pike. The observed level of activity was surprisingly high considering the small size of the lake. While pike is believed to be a sedentary ambush predator (Eklöv 1992; Craig 1996), studies have revealed that pike also utilise active hunting strategies (Turesson & Brönmark 2004) and regularly perform active movements (Diana 1980; Jepsen et al. 2001; Kobler et al. 2008). The present study corroborates these studies by showing that the pike on average moved 621–1248 m·day^{-1}.

Variation in water temperature is an integral part of the seasonal variation in lentic ecosystems and may influence pike activity (Casselman 1978). However, in longitudinal field studies, it is often impossible to isolate the effect of water temperature from other changing environmental factors that also may affect pike activity, for example, photoperiod and turbidity. Therefore, in the present study, potential effects of water temperature on pike activity were not statistically tested *per se* but were included in the combined effect of the periods (Table 1). Thus, as water temperature declined monotonically through the periods whereas pike activity remained high, the present study indicates that, although inconclusive, water temperature and ice cover seem not to affect pike activity levels. Previous studies report variable seasonal effects on pike activity, including decreasing, unchanging and increasing overall activity levels when comparing summer and winter (Diana et al. 1977; Cook & Bergersen 1988; Jepsen et al. 2001; Koed et al. 2006; Kobler et al. 2008). However, a direct comparison of these studies, including the present, is difficult because of the differences in applied methodology. Moreover, each study site comprises a unique suite of complex biological, environmental and physical variables and interactions, each of which the pike potentially respond to or utilise in variable ways. Indeed, considerable between-lake variation in pike behaviour and response to environmental stimuli has previously been documented (Jepsen et al. 2001; Andersen et al. 2008). In this respect, Lake Gosmer differs from the majority of study sites [e.g., Lac Ste. Anne (Diana 1980), Eleven Mile Reservoir (Cook & Bergersen 1988) and Lake Kleiner Döllnsee (Kobler et al. 2008)] in being smaller, more turbid and lacking submerged macrophytes. It is conceivable that the lack of submerged macrophytes could reduce the success rate of ambush attacks and oblige the pike to use more active hunting methods. However, Turesson & Brönmark (2004) found that pike are able to utilise sit-and-wait attacks even when exposed, by remaining motionless for extended periods of time followed by a sudden strike towards prey. Thus, macrophyte cover might not be a prerequisite for the pike to utilise sit-and-wait attacks. In that case, the relatively high turbidity of Lake Gosmer might increase the sit-and-wait success rate because the distance at which the prey detects the pike is reduced (Turesson & Brönmark 2007). However, the reduced detection range also influences the ability of pike to visually detect the prey and, thus, reduces the visual predator–prey encounter rate (Turesson & Brönmark 2007), which might impel the pike to increase swimming activity in search of prey. Inter-study differences aside, there seems to be consensus from field studies that pike maintain at least some activity through the cold seasons, even under ice cover (Diana et al. 1977; Cook & Bergersen 1988; Jepsen et al. 2001; Koed et al. 2006; Kobler et al. 2008). In contrast to this, Casselman (1978) found a strong correlation between swimming activity and temperature in laboratory settings, with optimum activity at approximately 20 °C and virtually no activity below 6 °C. In the light of Casselman (1978) and the general effect of temperature on physiological processes (Fry 1971), the observed pattern of high activity levels during the cold season may seem surprising. However, although decreasing water temperature affects the fish's maximum swimming speed (Claireaux et al. 2006), it does not render the pike immobile. Furthermore, the findings of Casselman (1978) are based on juvenile and subadult pike, whereas the present and other field studies (Diana et al. 1977; Cook & Bergersen 1988; Jepsen et al. 2001; Koed et al. 2006; Kobler et al. 2008) examined adult pike. Moreover, Casselman (1978) provided the pike food *ad libitum*, which might have reduced the pike's motivation for movement. Additionally, Schwalme & Mackay (1992) suggest that the majority of fatty acids accumulating during winter in recrudescing pike ovaries originate from dietary nutrients consumed during ovarian recrudescence as opposed to transfers from somatic tissue. This indicates that at least mature female pike forage and, thus, are active, year-round, to optimise reproductive success. Supporting this, Diana (1979) found that both female and male pike from Lac Ste. Anne forage through winter, although daily rations were low.

A distinct diel pattern was evident in all periods, showing differences in activity between night and day. The shifts from night to day, and vice versa, were often accompanied by a peak in activity (Figs 2 and 3). Furthermore, timing of these shifts between night and day levels of activity followed the times of sunrise and sunset. Previous field studies have found diel variations in activity with main findings comparable to the present study (Diana 1980; Cook & Bergersen 1988; Jepsen et al. 2001; Beaumont et al. 2005; Kobler et al. 2008) and, although variation is ubiquitous, there seems to be consensus that pike activity is low at night, higher at daylight and peaks at twilight. This diel pattern substantiates the notion that pike generally is a visual predator feeding during the crepuscular periods (Casselman 1996), even though it should be noticed that movement *per se* might not be a good indicator of pike foraging periods as pike are known to utilise a sit-and-wait foraging strategy (Eklöv 1992; Craig 1996). Nevertheless, as discussed above, pike may also use active hunting strategies (Turesson & Brönmark 2004) and the low-light conditions in twilight might promote these. Thus, the activity peaks at twilight could be related to increased forage intensity whereby the pike could exploit a competitive advantage as stated by the 'twilight hypothesis' (Pitcher & Turner 1986). According to this, the pike have an advantage in low-light conditions as the prey react later than in full light, when approached by a stalking pike (Pitcher & Turner 1986).

In spite of progressively reduced day lengths, the pike sustained a relatively high daily activity level throughout the periods (Fig. 1). This might be caused by increased activity during the illuminated hours of which there are some indications in Fig. 2. Temperate lake prey fish are known to display significant seasonal changes in behaviour including reduced activity and altered habitat utilisation (Jacobsen et al. 2004; Skov et al. 2011). This could, although speculative, be a reasonable explanation for the increased pike activity in winter as the pike may have to increase movement in order to encounter prey.

Temperate freshwater lakes are often considered to be inactive and static ecosystems during winter (Salonen et al. 2009). However, as shown in the present study, the natural apex predator in these lakes remains active in cold water and even under ice. It is well known that pike can have a profound structuring effect on lower trophic levels in freshwater lakes during summer, through cascading top-down effects exerted on the prey fish community (Prejs et al. 1994; Berg et al. 1997; Skov et al. 2011). Besides the direct effects on prey fish abundance and population structure, mediated through predation, indirect effects on the prey fish community are known. These include changes in morphology (Brönmark & Miner 1992; Eklöv & Jonsson 2007), reduced activity (Lima & Dill 1990; Bean & Winfield 1995; Jacobsen & Berg 1998) and altered habitat utilisation (Jacobsen & Perrow 1998) underpinning the ecological importance of this species. The overriding factor driving this structuring effect is undoubtedly that the pike represents an instant threat to the prey fish. As visual cues from pike induce changes in prey fish behaviour (Mikheev et al. 2006; Martin et al. 2010) and the probability of pike–prey encounters, *ceteris paribus*, is positively correlated with pike activity, the magnitude of the structuring effect is likely linked to pike activity. Furthermore, as discussed above, at least mature female pike appear to be foraging year-round. As our study strongly suggests that pike remain active to the same extent summer and winter, it seems plausible that some structuring effects of pike on the prey fish community might also be present during winter. Supporting the hypothesis of a structuring effect during winter, Skov et al. (2011) found that in partially migratory prey fish communities, the individual propensity to migrate for predation refuge in connected streams during winter is positively correlated with individual size-related risk of predation from pike. However, more research, simultaneously studying the behaviour of pike and prey fish during winter, is warranted to further test the hypothesis of a year-round structuring effect.

The implementation of an ATPS to study the behaviour of a lentic fish species proved to be successful by yielding unprecedented time series on individual pike behaviour in natural settings. From this large data set, it is evident that the behaviour of pike is very variable both within and between individuals (data not presented here). As the present study and the applied statistical model focus on establishing and analysing general patterns in pike behaviour, it should be noted that these general patterns potentially enshroud this large variation. Furthermore, it should be noted that the presented results are based on the behaviour of pike in a single lake for which reason replicate studies should be carried out to test the generality of the findings. Nevertheless, the present study covers two consecutive years and although year-to-year variation was apparent, winter activity was high in both years. Furthermore, owing to the small size of the study lake, long unidirectional movements (i.e. >140 m), as have been found in larger lakes (e.g. Kobler et al. 2008), were impossible. Therefore, it is conceivable that activity could be at the same level or even higher in larger lakes. We acknowledge that the habitat constriction in PER1 both years caused by a thermocline rendering 17% of lake volume anoxic potentially compromises the between-period comparisons. However, as the reduction in available habitat was relatively small (17%), we believe that the additional information gained from including a period with warm water, relative to the other periods, outweighs the potential impediments.

In conclusion, the present study indicates that the activity of pike in a temperate lake remains high through the seasons from late summer to winter. These findings add to the accumulating evidence, suggesting that lentic ecosystems are less static and more active during the cold season than previously assumed. Furthermore, the findings suggest that the structuring effects of pike on lower trophic levels, which have been documented during summer, potentially can be present during the winter as well.

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Effect of anthropogenic disturbances on lake fish individual behaviour

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Effect of anthropogenic disturbances on lake fish individual behaviour

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Abstract

Recreational activities are increasing in nature. Such activities will interfere with the related ecosystem and eventually affect fish in various ways, potentially leading to suboptimal behaviour. The effect of angling and boating on roach, perch and pike behaviour was explored by high resolution tracking using an automatic acoustic telemetry system and transmitters with sub-minute burst rates. Two protocols of four hours disturbance were performed in the lake every third day for 32 days, boating with a combustion engine or boating + angling with artificial lures. Responses to disturbance were very species specific; Roach swimming speed were considerably higher during the four hours of disturbance compared to days with no disturbance with an immediate reaction when the engine started. Perch reacted most during the first hour, whereas pike seemed rather to lower activity level. There was no difference between boating and boating + angling, indicating that boating was the primary source of disturbance.

Introduction

Many recreational activities in nature are associated with water. Depending on intensity such activities, e.g. angling and boating, will interfere with the related ecosystem either temporarily or more persistently. Besides deterioration of the physical habitat, shore line or vegetation (Lewin *et al.* 2006), also wildlife can be affected. Especially the effect on birds due to shoreline deterioration and direct disturbance has been studied and proved to interrupt nesting, resting and feeding with implications for distribution and species richness (see Lewin *et al.* 2006).

Recently attention has been drawn to how underwater noise, especially from boats and vessels may affect fish behaviour (Graham & Cooke 2008; Popper & Hastings 2009; Picciulin *et al.* 2010; Slabbekoorn *et al.* 2010; Purser & Radford 2011) and the need of understanding possible implications on ecosystem level (Slabbekoorn *et al.* 2010). Recreational boating and angling is increasingly popular and the effect on fish is highly relevant for management purposes. Angling activity is done both from the shore, where it can induce damage or disturbance to the riparian habitat and wildlife (see Lewin *et al.* 2006), and from boats. Besides being noisy, approaching boats might also disturb fish by sight or by propulsion waves (Wolter & Arlinghaus 2003).

Fish might react to disturbances in the water by a change in behaviour, for instance changing activity level or habitat use, the behavioural response to a moving boat being similar to the response to an approaching predator (Lima & Dill 1990). These changes in behaviour might be energetically expensive and lead to suboptimal performance. Hence, a change in activity e.g. flight or fright response is costly both in the amount of energy used but also in term of time lost from important behaviours (Brown *et al.* 2005; Cooke *et al.* 2003). This could mean reduced focus on foraging, as shown for sticklebacks (*Gasterosteus aculeatus*) (Purser & Radford 2011) as well as distracting attention from important mating behaviour or territoriality (Sebastianutto *et al.* 2011). Eventually attention shifts due to disturbance might lead to increased vulnerability to predators by preventing anti predator behaviour or by accidental dislocation to higher risk habitats (Chan *et al.* 2010). Noise linked to boating can affect fish hearing, for some species of fish this can be detrimental since they use hearing to obtain information from conspecifics, about predators or food or during mating (Amoser & Ladich 2005.).

The behavioural reactions are coupled to physiological processes, i.e. the immediate raised swimming speed as a fright reaction is facilitated by a raise in stress hormones (Lima & Dill 1990; Brown *et al.* 2005). In line, most studies of fish reactions to boat disturbances have been conducted by studying fish physiological reactions in the laboratory. For instance largemouth bass (*Micropterus salmoides* (L.)) has shown raised cardiac output (increase in heart rate and decrease in stroke volume) (Graham & Cooke 2008) when exposed to boat noise in an aquarium setup. Ship noise has also proved to be a stressor on three freshwater fish species by inducing a higher cortisol secretion (Wysocki *et al.* 2006).

Some studies have combined video recordings or sound recordings in the wild with experimental approaches in the lab (Boussard 1981; Wysocki *et al.* 2006), or vice versa, studying fish reactions in the wild by replaying boat noise recordings (Picciulin *et al.* 2010) showing slight changes in activity or avoidance of noise. However, there is a lack of knowledge on free swimming fish in the wild and their reactions to disturbances (Graham & Cooke 2008; Slabbekorn *et al.* 2010).

A prerequisite for studying fish behaviour when disturbed by humans and to show any effect on fish in their natural environment is the possibility to determine the undisturbed situation, hence being able to monitor the fish by a non-disturbing method. Monitoring of fish normally imply fishing gear, hydro acoustics or conventional telemetry, which all involves a manual monitoring protocol which in itself cause the fish to be exposed to disturbance. To circumvent this we used an automatic acoustic telemetry positioning system which makes it possible to study fish behaviour with no concurrent interference of the natural situation. The system renders high resolution data on fish positions with high spatial accuracy several times a minute and a large number of fish can be positioned simultaneously (see Niezgoda *et al.* 2002). This way the effect of any changes induced to the environment could be monitored with increased precision compared to traditional telemetry and without unintentionally disturbing the fish.

The present study aimed to assess the effect of recreational boating and rod angling on fish behaviour in a small lake on a short time scale. It was hypothesized that going with a low speed combustion engine would have a direct effect on fish behaviour by altering fish activity levels. Additionally, it was hypothesised that angling with artificial lures would interfere with fish behaviour, having in mind that anglers often experience fishing success to decrease a while after extensive fishing effort (Askey *et al.* 2006). Since angling cannot be done without shore activity or boat activity, the effect of angling from a boat is tested, presuming that angling might add to the disturbance effect of the boat.

The experiment comprised three species of common lacustrine fish in Northern Europe, roach (*Rutilus rutilus* (L.)), European perch (*Perca fluviatilis* L.) and northern pike (*Esox lucius* L.). The three species were expected to react to disturbances to a variable degree due to their inherent behavioural patterns.

Methods and Study area

The study was conducted in the small Lake Gosmer (area approximately 1 ha, max depth 8 m) in Mid Jutland, Denmark. The lake is on private land and there is no public access. The lake is eutrophic (secchi depth annual mean 1.2, range 0.4-2.4) and has no submerged vegetation, but dense stands of reeds (*Typha latfolia* L.) along most of the shoreline interrupted by a single bed of water lilies (*Nuphar luthea* (L.)). The north-western shoreline of the lake is shaded by overhanging trees. The fish community consisted of pike, roach and perch as well as a small population of stocked eel (*Anguilla anguilla* L.).

The tracking/telemetry system

A CDMA based acoustic positioning telemetry system (Lotek MAP 600; Lotek Wireless, Inc.) was installed in the study lake. The system works with eight hydrophones situated around the lake along the shoreline to ensure to cover the lake area in the most optimal way (see Niezgoda *et al*. 2002; Cooke *et al*. 2005). The hydrophones were positioned ca. 1m below water surface and connected to the receiving data logging station on the bank side by underwater cabling. Positions of fish were logged and data processed by use of proprietary software (BioMap v. 2.1.12.1; build 2.633; Lotek Wireless, Inc.), each position based on three or more synchronized hydrophone recordings (see Niezgoda *et al.* 2002). The raw positions were subsequently filtered using a Hidden Markov model based approach (Pedersen *et al.* 2008), in order to objectively remove outliers inevitably produced by the proprietary software (see Niezgoda *et al.* 2002).

Fish

Twenty-one pike (total length 48-100 cm) caught by angling, trammel net or electrofishing from March 2009 to September 2010 and concurrently tagged were employed in the study. In September 2010, 21 roach (total length 16.6 – 24.3 cm) and 23 perch (total length 16.2-24.0 cm) were caught in the lake by angling with barbless hooks. All tagging of fish followed the same surgical procedure. After capture the fish were kept in a keeping box (large, submerged, perforated wooden box) and before tagging they were anesthetised in a solution of MS-222. Individual pike were tagged with an acoustic transmitter (MAP TP11-25, weight in air; 11g; Lotek Wireless Inc.) with a guaranteed battery life of 889 days and a burst interval of 45 sec. Roach and perch were tagged with MAP 6-2 transmitters (weight in water 0.9 g; Lotek Wireless Inc), with a guaranteed battery life of 42 days and a burst interval of 30.7 sec. The transmitter was surgically implanted in the buccal cavity close to the pelvic girdle. The fish were also tagged with a Passive Integrated Transponder (PIT; 11.9 mm long; 1.9 mm diameter; Texas Instruments) for simple identification. The incision was closed with one or two separate sutures (Viacryl absorbable, ETHICON, Johnson & Johnson). Tagging procedure lasted 3-5 min and fish were allowed to recover from anaesthesia in a separate tank containing aerated lake water before released into the lake.

Experimental protocol

The tagged fish were left undisturbed in the lake for minimum seven days before the experiment was carried out from 14 of September to 11 of October 2010. A treatment protocol was performed with two types of disturbance, either boating (with a four stroke outboard combustion engine, 4HP) or boating+angling. Boating was in both cases done in a predefined pattern simulating the boating activity during angling; i.e. engine was turned on, boat moved 1-2 min. (63.5 ± 21,1 m) to a pre-determined position (position of a hydrophone), engine turned off again. Every 15 min. the boat moved to another pre-determined position and after 4 hours most of the lake was covered in a star-like pattern. During the boating+angling treatment the boat moved in exactly the same pattern, but when pausing at the fixed position, two persons angled by casting a small artificial lure (3.5 g standard spinner) in a circular pattern until next move. The boat was mounted with a transmitter (Map 6-2, burst rate 2.5 sec.; Lotek Wireless Inc.), in order to accurately follow the boat track.

Disturbance took place from 9.00 to 13.00 every third day during the study period, alternating between the two types of disturbance. Five days (replicates) of each disturbance protocol were performed and in-between the system was left undisturbed. The day before each disturbance was used as controls representing the undisturbed natural situation (n=10).

Data analysis

Fish behavior was quantified using swimming speed (cm * s^{-1}) calculated for all instances where the maximum obtainable temporal resolution was achieved; i.e. instances where the time between two consecutive positions was equal to the transmitter burst interval (= 30 sec). These values were summarized as day specific individual hourly median speeds, thus yielding 24 hour time series on the individual median swimming speeds. These time series were analysed using generalized additive mixed models (GAMM), one model for each species, following the procedures in Zuur *et al.* (2009) and Wood (2006). In each model swimming speed was entered as dependent variable and WaterTemp, Total Length (TL), Treatment (i.e. control, boating and boating+angling), TimeOfDay (TOD) and the Treatment*TOD interaction as independent variables. FishID was entered as a random variable, thereby imposing a compound symmetry correlation structure allowing the repeated measures made on each fish to be correlated (random intercept model). Furthermore, a first order autoregressive autocorrelation (AR1) structure was included to model the temporal correlation between observations made on each fish. Relevant variance structures were tested in each model to account for variance heteroscedacity. The additive model framework was chosen to model the non-linear effect of TOD on swimming speed by employing smoothing functions. Cyclic cubic regression splines were used to model the cyclic nature of TOD. For each species, the optimal number of degrees of freedom for the smoothing functions was determined using AIC-values (Keele, 2008). To model the Treatment*TOD interaction separate smoothers for each level of the Treatment factor (i.e. control, boating and boating+angling) were fitted. Additionally, reduced models with two smoothers (one for control and one for boating and boating+angling combined), models with a single smoother (one for control and one for boating and boating+angling combined) and models excluding TOD were fitted. By comparing these reduced models to the full model using AIC values, we tested whether there was an overall effect of Treatment on fish swimming speed. Model selection was based on Akaike Information Criterion (AIC). The hourly median swimming speeds were transformed (log(y + 0.01)) to meet model assumptions of normality. Additionally, individual fish length was centered to the mean length of all fish included in each model to make model parameters representing a fish with TL = mean length instead of a fish with TL = 0 cm.

The applied GAMM approach allowed a robust assessment of whether there was an overall effect of Treatment on fish swimming speed by utilizing the entire dataset on each species to model and compare daily patterns. However, as this approach utilized the entire dataset it was less appropriate for detecting sudden changes in the response variable such as those potentially induced by the treatment protocol. To assess whether such instantaneous effects of Treatment were present, we tested the significance of the Treatment within each TOD. Thus, for each species, 24 identical linear mixed effect models (random intercept) were fitted using log(Speed + 0.01) as response variable, WaterTemp, TL and Treatment as explanatory variables and FishID as random variable. The significance of Treatment in each model was tested using likelihood ratio test according to Zuur *et al.* (2009). To correct for the multiple tests, Bonferroni corrected p-values was calculated as $p_{Bon} = 0.05 / 24$ (Quinn & Keough, 2002).

Changes in spatial distribution of fish caused by disturbance was described by mapping all positions of fish during 9-13 on days without disturbance and days with disturbances, both boating and boating+angling.

Results

Roach was significantly affected by disturbance with an overall change in the daily pattern of hourly activity (swimming speed) compared to control days (Figure 1a). There was no significant difference between boating disturbance and boating+angling disturbance (2 smoothers better than both 3 and 1); both disturbances caused increased swimming speeds (Table 1; Figure 1a).

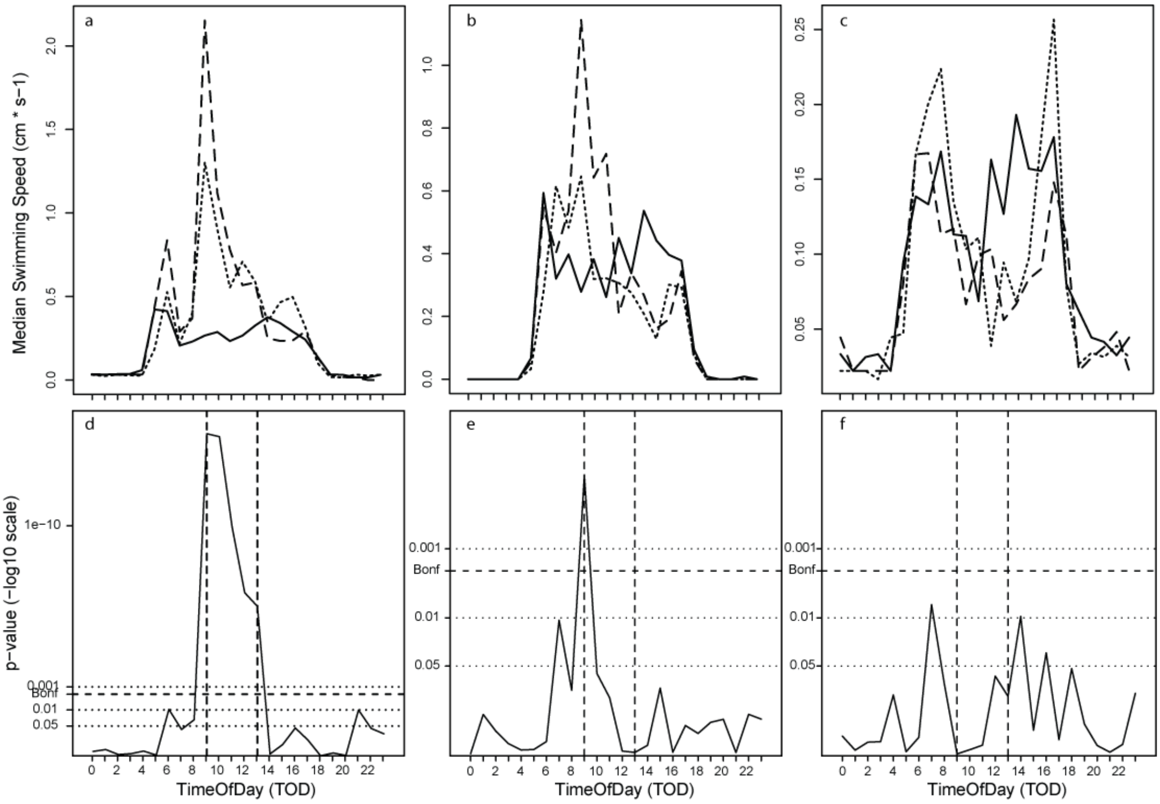

Fig. 1 Upper row: overall median of individual hourly median swimming speed (cm * s^{-1}) for roach (a; n = XX), perch (b; n = YY) and pike (c; n = ZZ) during days of control (solid, n = 10), boating (dashed, n = 5) and boating + angling (dotted, n = 5). Lower row: statistical effect of treatment per hour (-log10 transformed p-values) for roach (d), perch (e) and pike (f). See main text for further explanation. Notice different scales on the y-axis in both rows. Vertical dashed lines indicate start (TOD = 9) and end (TOD = 13) of the disturbance protocol.

There was an immediate effect of disturbance on roach swimming speed (highly significant; p < 1E-14) from 9:00, when disturbance was initiated and throughout the disturbance period (all p-values well below the Bonferroni corrected threshold, Figure 1b). There was no difference in swimming speed between disturbance days and control days in the hours before and after disturbances (Figure 1b). The increased activity leveled off during the disturbance period, and no effect was seen after disturbance had ended. Throughout the disturbance periods roach reacted instantaneously to boat noise by increasing swimming speed during the 2-3 min. the boat engine was running, revealed as an increase in swimming speed every 15 min. (Figure 2).

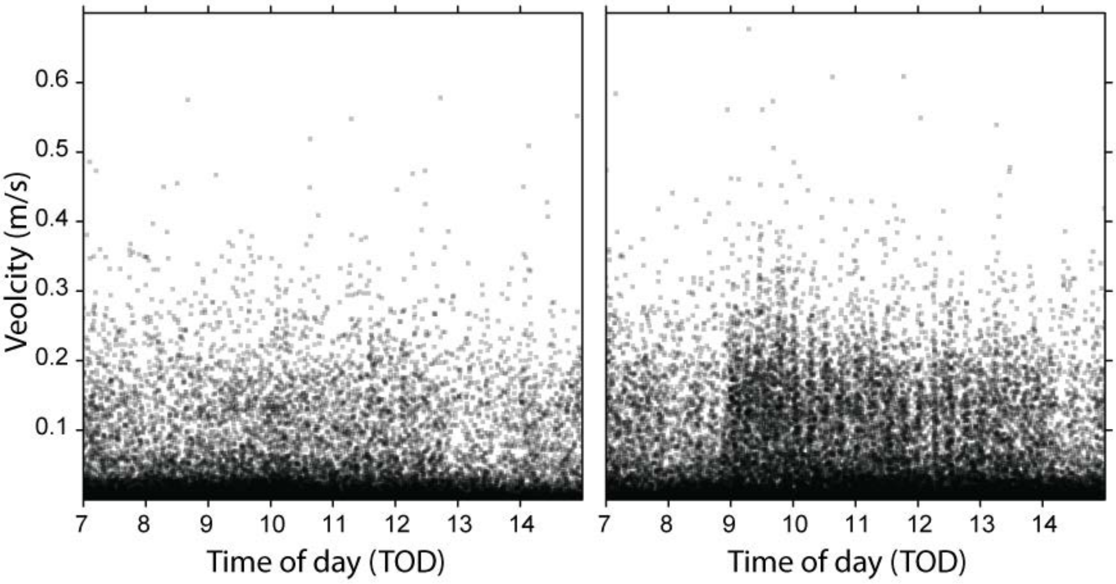

Fig. 2. Individual swimming speed for each roach during control days (left) and days with boating from 9-13 (right).

The model revealed a tendency ($p > 0.05$) to positive effects of length (TL) and water temperature on roach swimming speed (Table 1).

For perch there was no statistical evidence for an overall disturbance effect on daily pattern of swimming speed (Table 1; one smoother for control, boating+angling was better (lower AIC) than three or two smoothers), see Figure 1c. However, non-significant tendencies towards positive effect of disturbance on swimming speed were apparent (Figure 1c). Testing each hour separately revealed an immediate effect at 9:00, when disturbance started, well below Bonferroni corrected p-threshold (see Figure 1d). There was no effect of length (TL) and water temperature on perch swimming speed.

The model output for pike revealed no evidence for overall effect of disturbance (Table 1; one smoother better than two or three) see Figure 1e. However, there were indications of boating and boating+angling causing pike activity to be lowered; but this was inconclusive. The activity for pike was highly variable (compared to roach and perch) which potentially clouded detection of a disturbance effect (Tabel 1) . The model further revealed significant effects of water temperature (negative effect) and length (TL) (positive effect) on pike activity (swimming speed). There were no indications for any effect within disturbance period, when testing each hour separately (Figure 1f).

Spatial distribution of roach changed from a more diverse pattern in the lake under control conditions to a more condensed pattern in the middle part of the lake with avoidance of certain spots of previous preference, when disturbance took place (Figure 3 a-b). Especially, roach avoided the spots where the boat anchored up e.g. in the middle of the lake, where the boat anchored at a floating data logger station.

Perch and pike did not seem to change their overall distribution in the lake due to disturbance (Figure 3c-f). Perch as well as pike concentrated in certain areas; perch seemed to prefer the edge of the deeper part of the lake, whereas pike occupied the littoral zone also in the shallower parts of the lake.

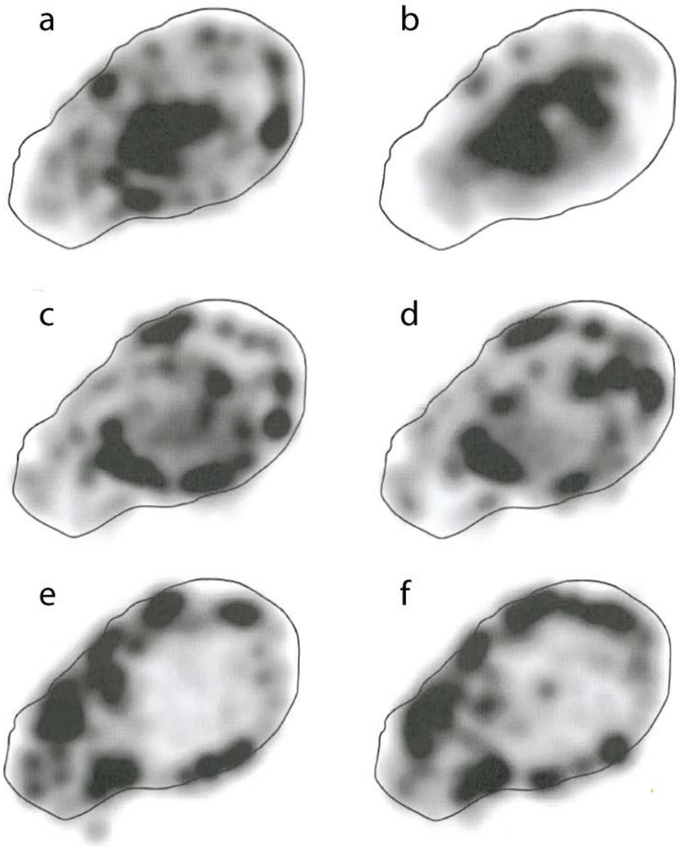

Fig. 3. Habitat distribution during control days 9-13 (left) and during days of disturbances 9-13 (right) for roach (a-b), perch(c-d) and pike (e-f). Kernel density plots – the darker, the more positions.

Table I. Model summaries for the applied GAMMs for roach (a), perch (b) and pike (c). The effect of Treatment was tested using Akaike Information Criterion (AIC) by comparing a full model (three smoothers: Control, Boat, Boat+Angling (B+A)) to models with two smoothers (Control, Treat (Boat and Boat+Angling combined)) and models with a single smoother. Smoothing terms are indicated by f(). Models with best fit are highlighted in bold. Effects of Temp and Length were tested using likelihood ratio tests; significant effects are highlighted in bold. Note that parameter estimates and standard errors (s.e.) are on the log(y+0.01)-transformed scale.

	Parameter	Estimate (s.e.)	AIC	ΔAIC	P
a) Roach					
Full Model			33599.46	-	-
	Intercept	-2.378 (0.257)	-	-	-
	Temp	0.0221 (0.0196)	33600.21	0.75	0.0973
	Length	0.00367 (0.00237)	33600.65	1.19	0.0738
	Treatment	-	-	-	-
	Control	0	-	-	-
	Sail	0.280 (0.0589)	-	-	-
	Fish	0.2549 (0.059)	-	-	-
	f(TOD; Control, Boat, B+A)	-	-	-	-
Reduced models	**f(TOD; Control, Treat)**	-	**33555.67**	**-43.79**	-
	f(TOD; 1 smoother)	-	33591.21	-8.25	-
	f(TOD) removed	-	35638.20	2038.74	-
b) Perch					
Full Model			33644.82	-	-
	Intercept	-2.446 (0.304)	-	-	-
	Temp	0.0149 (0.0212)	33643.61	-1.21	0.3735
	Length	0.00415 (0.00676)	33643.12	-1.7	0.582
	Treatment	-	-	-	-
	Control	0	-	-	-
	Sail	0.137 (0.0697)	-	-	-
	Fish	0.0797 (0.0696)	-	-	-
	f(TOD; Control, Boat, B+A)	-	-	-	-
Reduced models	f(TOD; Control, Treat)	-	33605.39	-39.43	-
	f(TOD; 1 smoother)	-	**33566.69**	**-78.13**	-
	f(TOD) removed	-	35635.90	1991.08	-
c) Pike					
Full Model			29296.43	-	-
	Intercept	-1.599 (0.344)	-	-	-
	Temp	-0.05254 (0.0250)	29307.70	11.27	**0.0003**
	Length	0.00373 (0.00105)	29313.05	16.62	**<0.0001**
	Treatment	-	-	-	-
	Control	0	-	-	-
	Sail	-0.0979 (0.0746)	-	-	-
	Fish	-0.0463 (0.0773)	-	-	-
	f(TOD; Control, Boat, B+A)	-	-	-	-
Reduced models	f(TOD; Control, Treat)	-	29280.61	-15.82	-
	f(TOD; 1 smoother)	-	**29261.23**	**-35.2**	-
	f(TOD removed)	-	29848.64	552.21	-

Discussion

The study confirmed our expectations, that boating with a combustion engine will affect fish behaviour in a lake, however with a species specific outcome. The reaction from roach was highly significant and instantaneous, increasing swimming speed, when disturbance started. When disturbance ended roach returned to normal swimming speeds during the first hour, so no long term effect of disturbance was apparent. None of the species showed any additional disturbance effect when boating was combined with angling with artificial lures, suggesting that boating was the main disturbance factor of the two. It should be noted though that the present study design does not allow for conclusions on the isolated effect of angling, since angling was only conducted in combination with boating. Klefoth *et al.* (2011) concluded that there was no indirect effect of angling on pike activity levels, however, they were monitoring pike in angling and non-angling areas by manual tracking from a boat. Other studies of angling effect on fish behaviour have focused on the direct effect of catching fish and the rationale of catch and release (Klefoth *et al.* 2008; Arlinghaus *et al.* 2009).

The present study suggests that reaction to disturbance is species specific. Compared to roach, perch showed a large variation in activity patterns during daytime with a less clear overall reaction to disturbance compared to control days. It was, though, apparent that perch increased swimming speed during the first hour, but hereafter, there was no effect on activity levels of the continued disturbance. It could be explained by the perch getting used to the noise during the first hour; habituation to constant noise (Gaussian noise) has been described for some species (Brown *et al.* 2005) and roach and rudd (*Scardinus erypthropthalmus* L.) showed some habituation to noise bursts (Boussard 1981). Alternatively, perch were seeking areas away from the disturbance, though these might be transitory, since boating took place in most of the lake during the four hours. The deeper parts in the middle of the lake could form a refuge from boat noise, however, even though the maps of distribution indicated that perch might prefer other spots during disturbances, they did not move to the middle of the lake.

Pike did not react to boating or boating + angling by increased activity levels. It rather seems that the median swimming speed decreased during the last part of the disturbance period, indicating that pike might have decided for quiet littoral zone parts and deceased activity as a response to boating. Maps of distribution confirmed that pike stayed in the littoral with no obvious change in preferred area. Pike has a very large individual variation in behaviour (Jepsen et al. 2001; Kobler et al 2009); whereas some pike remain rather inactive during daytime probably due to its ambush hunting style, others are more active, adopting a more stalking hunting mode (Jepsen et al. 2001). The slight decline in median swimming speed might imply that the more active individuals ceased their hunting activity during disturbance.

Roach is a pelagic fish species with a high swimming capacity preferring the surface waters during summer (Eklöv & Persson 1995; Brosse *et al.* 1999; Tudorache *et al.* 2008; Linløkken *et al.* 2010). This behavioural feature might render roach more sensitive to disturbances and imply a more active fleeing behaviour. Species specific hearing sensibility might also explain why roach reacted so abruptly and significant to boat noise (Wysocki *et al.* 2006; Boussard 1981). As other cyprinids, roach is a hearing specialist, able to hear far lower intensities and more frequencies of sounds than perch and pike, both categorized as hearing generalist (Amoser & Ladich 2005). The fact that roach responded more substantial to disturbance might also be explained by species specific differences in stress responses (Pottinger 2010); cyprinids species have shown higher and more sustained stress responses, i.e. induces plasma cortisol and glucose levels (indicator of stress-induced energy mobilization), compared to salmonids (Pottinger 2010).

The overall effect of the four hours of disturbance ceased less than an hour after disturbance stopped. This is in accordance with Graham & Cooke (2008), who found that the effect of a combustion engine on cardiovascular output lasted up till 38 min; this effect was obtained after engine noise for only 60 sec. Most studies of responses to boat noise are done in lab conditions (see Graham & Cooke 2008 for review) where fish are in close proximity to the source of noise during the whole period, experimental condition not allowing for fleeing from the source of disturbance. Hence, the level of physiological stress or loss of hearing is likely to be unnaturally high. In our study, fish likely fled away from the boat when the engine started running and the individuals in closest proximity to the boat might have been eliciting the highest immediate swimming speed simultaneously with the boat start. This was not tested further due to the 30 sec. burst interval of the transmitters, which is inadequate for studying detailed fleeing behaviour. The fact that the fish seemed to avoid the areas of noise would affect the level of physiological stress and temporary hearing losses.

In the present study the boat engine was running for 2-3 min. every 15 min. Though individual variation in proximity to the noise source might cause individual levels of physiological stress (Graham & Cooke 2008), it is rather feasible that fish might not return to normal physiological state in-between boat movements.

Graham and Cooke (2008) found the effect of noise on largemouth bass cardiac output to vary according to boat type. Whereas canoe paddling showed some effect, electric trolling motors increased the magnitude of the effect and a 9.9 hp combustion engine showed the most extreme effect, underpinned by studies on high speed boats (Boussard 1981, Sebastianutto *et al.* 2011), so it is likely to conclude that combustion engine noise in our study is a main trigger. However, other stimuli such as sight of the approaching boat as well at the mechanical pressure from the moving boat (Wolter & Arlinghaus 2003) could influence fish behaviour. The fact that roach actively avoid the location of boat anchoring, most explicitly seen in the centre of the lake insinuates that sight of the boat or, most likely, a prolonged effect of the short time engine noise was operative as well.

The abrupt increase in swimming speed provoked by the boat, resembling flight response to potential danger, will both induce physiological changes (Brown *et al.* 2005) and have energetic costs, if the fish is forced to move faster than routine swimming speed (Forstner & Wieser 1990). Stress response is not only costly in terms of raised metabolic costs but also in lost time for important activities as feeding, mating and territoriality (Brown *et al.* 2005; Purser & Radford 2011; Sebastianutto *et al.* 2011). Individual fitness is likely to decrease along with repeated and extended disturbances. In the present study disturbance was of limited periods, and the fish might be able to cope with this and do compensatory feeding when disturbance ends. Nevertheless, the results imply a potential large negative effect on fish behaviour in lakes with constant disturbance from recreational boating and fishing as well as other activities as vessel traffic and water piling.

In general, results from roach indicate that the chosen approach seems to be appropriate to detect treatment effects if any were present. Precaution should be taken, when concluding on results from a single lake, as well as for the size of the lake in the present study; it is likely that disturbance effects would be less in a larger or deeper lake, where fish might be able to retreat to areas out of range of noise disturbance. The capture and tagging of fish is not believed to affect results since the procedure has been evaluated in earlier studies (Jepsen *et al.* 2002) as well as in previous experiments carried out in Lake Gosmer, where a large amount (>200) of fish have been tagged over more years with no observations of negative effects of tagging (mortality, predation, abnormal behaviour).

In perspective, human recreational activities are likely to disrupt fish normal behaviour with possible implications for individual fitness as well as for the freshwater ecosystem, if this pressure is intense. Predator prey interactions can be a driving force in structuring lake ecosystems with cascading effects down to lower trophic levels (Carpenter & Kitchell 1993): The species specific behavioural alterations due to anthropogenic disturbance, as seen in the present study, might uncouple these interactions with consequences on ecosystem level.

Recreational activities and noise from other anthropogenic sources is increasing in nature (Slabbekoorn *et al.* 2010; Popper & Hastings 2009), hence, more focus on this is needed. The fact that some fish species are very sensitive to boat engines underlines the need for managing recreational activities on lakes in time and space.

The present study shows that fish not only react by physiological stress symptoms as shown by several authors in the lab (e.g. Wysowski *et al.* 2006; Graham & Cooke 2008) but also by changing behaviour. Hence, this is the first study to report on detailed behavioural response of free swimming fish in nature. The possibility of studying high resolution behaviours in both disturbed and natural situations by use of the acoustic positioning system made this kind of study possible and seems promising for studying anthropogenic impacts. Further details of immediate response and proximity to the source of noise could be possible by use of transmitters with shorter burst interval, though this is a trade off with longevity of study periods depending on fish size.

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Effects of angling and manual handling on pike behaviour investigated by high resolution positional telemetry

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Effects of angling and manual handling on pike behaviour investigated by high resolution positional telemetry

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Abstract

Anthropogenic disturbances such as angling and manual handling may have long term effects on the behaviour of pike (*Esox lucius* L.), an ecologically important species. Using automatic continuous high resolution positional telemetry, this study compared the swimming activity levels of handled and unhandled pike in a small lake. Pike pre-equipped with acoustic transmitters were angled and exposed to a handling protocol including measurements of length and mass. Not recaptured pike constituted an unhandled control group. Results demonstrated that the handling protocol caused temperature dependent changes in pike activity, with higher temperatures leading to lower activity of the recaptured pike. The effects however, were transitory and not detectable after 48 hours post release. These findings indicate that pike are relatively resilient to handling and quickly resumes pre handling activity.

Introduction

Time series information on fish positional behaviour and activity can be generated by implementing a variety of technologies (Lucas & Baras 2000). In recent years, the use of positional telemetry on free-ranging fish has enabled accurate quantification of changes in the use of space over time on an individual level (Lucas & Baras 2001). Telemetry has been utilized to study various aspects of fish biology (e.g. migration, survival and behaviour (Svendsen et al. 2004; Aarestrup et al. 2009)) as well as anthropogenic impacts on fish behaviour and survival (e.g. effects of angling and habitat alterations (Koed et al. 2006; Thorstad et al. 2008; Arlinghaus et al. 2008)). The strength of positional telemetric data is dependent on both the spatial precision and the temporal resolution of the data. Recent technological advances have increased both parameters allowing for continuous high resolution monitoring of tagged fish (Ehrenberg & Steig 2003; Cooke et al. 2005).

Most telemetric studies assume that any adverse effect of the tagging protocol on the behaviour of the focal fish is temporary and negligible after a given period of time (Koed & Thorstad 2001; Svendsen et al. 2004). Numerous studies have examined the effects of tagging and cover both physiological and behavioural aspects (e.g. Moore et al. 1990; Jepsen et al. 2002; Jepsen et al. 2008). Typically, these studies include a protocol describing the acquisition (e.g. by angling, netting or electro fishing) and handling (e.g. measurements of length and mass) of the fish prior to tagging and release. When based on angling, these fish acquisition protocols often resemble the catch-and-release practices employed by many recreational fisheries.

Telemetric studies describing the effects of catch-and-release angling report variable responses of the angled fish including reduced activity (Gurshin & Szedlmayer 2004; Klefoth et al. 2008), hyperactivity (Thorstad et al. 2004) and abnormal behaviour (Makinen et al. 2000; Thorstad et al. 2003). The tracking protocols of this type of studies are frequently based on manual positioning of the fish, i.e. personnel actively approach the fish in order to pin point the location of the fish. (e.g. Jepsen et al. 2000). This approach sets a natural limit to the temporal and/or spatial resolution achievable without disturbing the fish. Furthermore, as manual tracking is labour intensive, the duration and frequency of tracking sessions are often restricted. To avoid these potential shortcomings, an alternative method (high resolution positional telemetry (Niezgoda et al. 2002)) was used in the present study.

Being a wide spread top-level predator in many freshwater ecosystems (Craig 1996) and a popular target for recreational and commercial fisheries (Pierce et al. 1995; Arlinghaus & Mehner 2004), the potential impacts of anthropogenic disturbances on pike (*Esox lucius* L.) must be well documented in order to facilitate qualified management of this resource. Management regimes of pike populations often include size specific restrictions on the removal of individuals for consumption, which in turn necessitates release of captured individuals outside the allowable size range. Previous studies on the effects of catch-and-release angling of pike have found transitory behavioural and physiological effects (Klefoth et al. 2008; Arlinghaus et al. 2009). However, the behavioural parts of these studies were based on manual tracking and, thus, were susceptible to the previously mentioned potential shortcomings. Furthermore, the studies did not include a control group of non-manipulated pike to account for whole system alterations (changes in temperature, water clarity, etc.) that could be causing behavioural changes.

Data for the present study originate from a long term study on pike behaviour in which pike were caught by angling or netting during five temporally separated sessions and equipped with acoustic transmitters. During these sessions some of the already tagged pike were inevitably recaptured. These recaptured pike were handled to simulate standard procedures normally undertaken in telemetric (and other) studies including careful de-hooking, weighing and short or prolonged keeping in tanks or other holding facilities. By comparing behavioural changes in already tagged individuals before and after recapture with non recaptured tagged conspecifics (control group), this study evaluates the effects of a non-tagging handling and angling protocol on the post handling behaviour.

In summary, the main objective of the present study was to assess how the angling and handling protocol, comparable to what is often used when acquiring wild fish for research purposes, on its own, influences the activity of pike. This was done using automated positional telemetry allowing a high resolution comparison of activity just prior to and immediately following handling events.

Materials and methods

Study area

The study was conducted in a small Danish eutrophic lake (Lake Gosmer; 55°55´42 N, 10°10´50 E; wetted area approximately 1 ha; maximum depth 8 meters; total phosphorous 0.42 mg/l; mean secchi depth 107 cm in study period, fig. 1). The fish community consists of pike, perch, *Perca fluvaitilis* L. and roach, *Rutilus rutilus* (L). Submerged vegetation is restricted to a single bed of water lilies, *Nuphar lutea* (L.) along the southern shore. Narrow but dense stands of emergent macrophytes, *Typha latifolia* L. cover the majority of the shoreline apart from the northern shore which is shaded by overhanging trees.

Initial fish capture and tagging procedure

A total of 26 pike (mean length (SD) = 64.3 cm (13.9); mean weight (SD) = 2.0 kg (1.4)) were angled (24 fish) or caught in trammel-nets (2 fish) in five 2 – 3 days fishing sessions from March 3 to September 28 2009. Angling was done using ordinary recreational fishing gear equipped with either live bait or artificial lures.

The fish were equipped with coded acoustic sensor (temperature and pressure) transmitters (Lotek MA-TP11-25; 11 mm diameter; 61 mm long; 11 g in air; 45 second burst interval). Maximum transmitter:fish weight ratio was 2.5 %. Emitted signals contained information on temperature and pressure alternating in a 1:1 ratio. Fish were anesthetized using a 0.5 mg/l solution of 2-phenoxyethanol. The transmitters were inserted into the body cavity through an incision approximately 50 mm anterior to the pelvic fins. To allow for easy subsequent identification of the tagged fish, they were additionally equipped with a PIT-tag (Texas Instruments, RI-TRP-RRHP, half duplex, 134 kHz, 23.1 mm long, 3.85 mm diameter, 0.6 g in air) inserted through the same incision. Following tag insertions, the incision was closed using two separate sutures. During the tagging procedure, an anaesthetic solution (0.25 mg/l) covered the head of the fish and continuously irrigated the gills. Tags and instruments were disinfected in ethanol (96 %) and rinsed in deionised water. Furthermore, total length (TL) and fish mass were recorded. Duration of the complete tagging procedure was approximately 5 minutes per fish. After full recovery (10 – 30 minutes), the fish were returned to the lake. All fish were released at the same position in the lake.

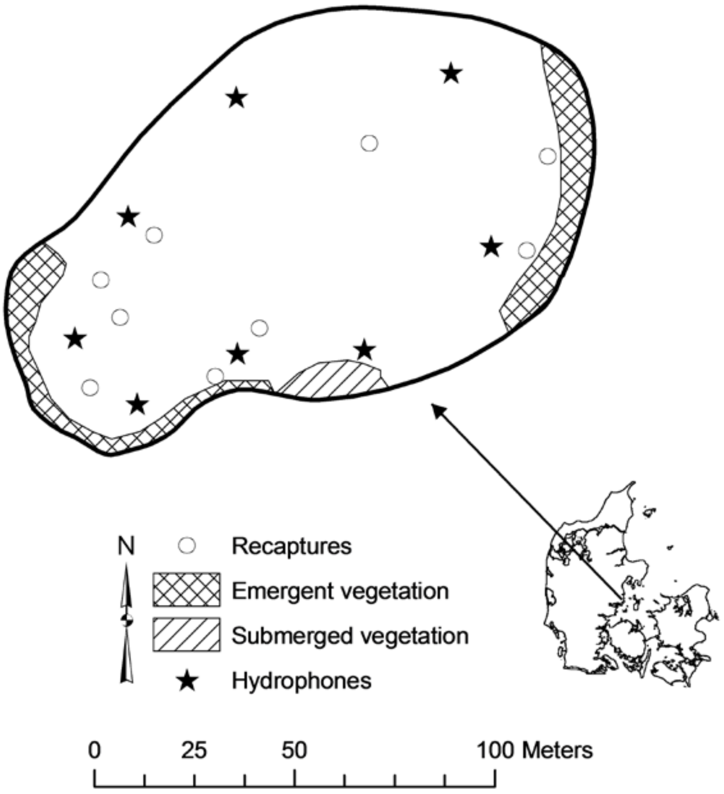

Fig. 1.Map of the study area showing positions of hydrophones (stars), recaptures (circles) and vegetation cover (hatched areas).

Experimental treatment

Nine tagged pike were recaptured in a total of 14 recapture events (one fish was recaptured five times and another fish was recaptured two times) during five angling sessions. The recapture events of the nine fish and the subsequent handling constituted the experimental treatment applied in the study. Only the first recapture event of each individual fish was included in subsequent analysis. Tagged fish not recaptured during a fishing session constituted the controls for that session (table 1). Newly tagged fish in a given session were not included in the analysis of that particular session. Handling of the recaptured fish involved quick de-hooking, identification and weighing in a net. Holding ranged from immediate release to prolonged holding in a plastic container (up to one hour) or a larger well box (up to 24 hours). Handling was conducted to minimize air exposure and overall handling time.

Table 1. Overview of number of recaptures and controls in the five fishing sessions.

Session	Centred timestamp	TEMP	#recaptures	#controls	#total
I	2009-03-20 09:15	5.4	2	8	10
II	2009-05-28 11:15	16.1	1	7	8
III	2009-09-07 02:05	16.0	1	6	7
IV	2009-09-27 15:29	14.7	3	11	14
V	2009-10-20 22:37	7.9	2	17	20
Total			9	49	58

Data acquisition and processing

The tagged pike were continuously monitored using a Lotek MAP_600 system with eight cabled hydrophones distributed along the shore. The system is based on code division multiple access (CDMA) enabling simultaneously high resolution tracking of a large number of tagged fish (Niezgoda et al. 2002). To acquire the calculated positions of the tagged fish, raw data obtained by the hydrophones were processed using company supplied software (BioMap v. 2.1.12.1; build 2.633; Lotek Wireless Inc.). In order to objectively reduce the effect of spurious observations unavoidably produced by the BioMap software, the raw positions were filtered and smoothed using a hidden Markov Model based approach with a t-distributed observation noise (Pedersen et al. 2008). The most probable track was then calculated by linking the means of the time marginal posterior distributions returned from the smoothing algorithm. All presented data are based on these most probable tracks. Average water temperature (TEMP) for each fishing session was calculated based on registrations recorded by a stationary temperature data logger positioned at the centre of the lake at one meters depth.

Data analysis and statistics

Moved distance per hour (DPH) of each fish was calculated as the accumulated distance between positions on an hourly basis. Mean number of positions per fish per hour was 34, thus DPH was on average determined as the total length of 33 line segments.

For each sampling session a centred timestamp (TIME0(control)) was defined as the middle time between first and last release of a recaptured fish when two fish or more was recaptured. In sessions where only one fish was recaptured TIME0(control) was defined as the time of release of that recapture. TIME0(control) for each session was assigned to all control fish in that session. For all individual recaptured fish TIME0(recap) was defined as the time of release. Maximum deviation of TIME0(control) from TIME0(recap) was 22 hours. Henceforth, the term TIME0 represents both TIME0(control) and TIME0(recap).

For all individuals, accumulated moved distances in the preceding (DIST_BEFORE) and following (DIST_DAY0-2) 48 hours relative to TIME0 were calculated as the sum of DPH in these periods. Similarly, accumulated distances in the following periods (all given as hours after TIME0) were calculated: 24 – 72 hours (DIST_DAY1-3), 48 – 96 hours (DIST_DAY2-4) and 72 – 120 hours (DIST_DAY3-5).

To obtain estimates of the individual differences in activity before and after TIME0, the relative change in accumulated distance was calculated for all combinations of individual fish and post TIME0 accumulated distances (DIST_DAY0-2, DIST_DAY1-3, DIST_DAY2-4 and DIST_DAY3-5) relative to DIST_BEFORE as e.g.: REL_DIST_DAY0-2 = (DIST_DAY0-2 – DIST_BEFORE) / DIST_BEFORE. These relative measures of activity differences were log10(x+1) transformed to meet the normality and homoscedasticity requirements of parametric analysis. The relative changes in accumulated distance of the recaptured group could be biased by the fact that foraging (and thus susceptibility to being caught) fish might exhibit different behavioural patterns resulting in increased activity compared to non-foraging fish. In order to test for this, a t-test was used to compare DIST_BEFORE for the recaptured and control groups assuming that no difference between the two would reflect similar pre-tagging activity patterns.

A General Linear Model (GLM) was applied to evaluate the effects of the handling protocol on the activity of the fish. The relative change in activity of individual fish was entered as the response variable, recapture (yes/no) as a fixed factor and TL and TEMP were entered as covariates. All interaction terms were included in the starting model and subsequently removed using backwards elimination ($P > 0.10$ to remove). The model was initially fitted to the response variable REL_DIST_DAY0-2 and afterwards the same model was applied to the remaining three response variables in order to ensure comparability between all four models. All statistical tests were done in SPSS 17.0 using $\alpha = 0.05$ as the level of significance.

Results

A single fish died immediately after tagging. During the study period (March 20 – October 25 2009) another two of the tagged pike died of unknown reasons (transmitters suddenly remained stationary with no subsequent movement). These fish are not included in the present study. Two other tagged individuals were predated by other tagged pike. These two are included in the analysis up to the predation event. Disregarding the tagging induced mortality, the total natural mortality rate during the study period was 16.7 % (4 out of 24 fish).

DIST_BEFORE of recaptures and controls was indistinguishable (t = 0.08, df = 56, P = 0.94) indicating that the relative changes in activity for the recaptured fish were not biased by systematically different behavioural patterns between the non captured control group and the recaptured individuals. Activity levels and patterns were highly variable in both the control and the recapture group both before and after TIME0 (table 2, fig. 2).

Table 2**.** Descriptive statistics of the accumulated moved distances in the periods before and after TIME0 for the control group and for the group of recaptures. All units are meters per hour. N(control) = 49; N(recap) = 9.

		DIST_BEFORE	DIST_DAY0-2	DIST_DAY1-3	DIST_DAY2-4	DIST_DAY3-5
Mean (SE)	Control	1615 (185)	1479 (205)	1473 (186)	1547 (176)	1670 (202)
	Recap	1650 (369)	991 (190)	991 (274)	1004 (311)	1146 (279)
Range	Control	67 – 6543	61 – 5902	73 – 5051	202 – 4993	123 – 7105
	Recap	409 – 3438	289 – 2078	276 – 2698	159 – 2697	205 – 2637

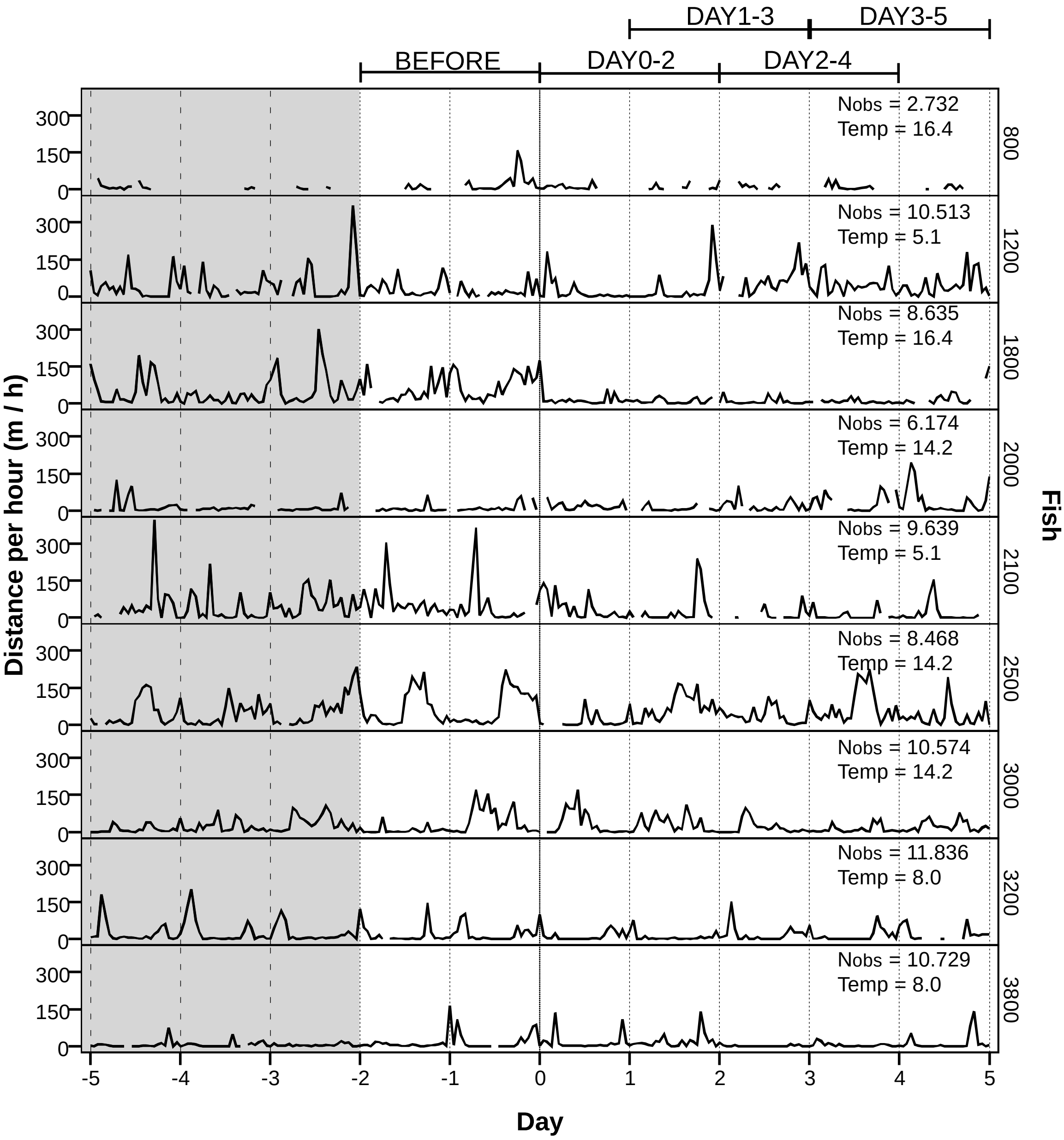

Fig. 2. Moved distance per hour for the nine recaptured fish five days before and after the recapture. Data preceding BEFORE (grey area) is not included in the analysis and shown for reference purposes only. Gaps in lines represents hours where one or none positions of the fish were obtained. Total number of positions (Nobs) in the ten day period of each individual fish as well as mean water temperature (Temp in degrees Celsius) in the period are given.

The GLMs revealed a significant effect of the RECAP*TEMP interaction on the relative change in moved distance observed in the first two periods (DAY0-2 and DAY1-3) but none thereafter (table 3). This interaction demonstrates that an effect of RECAP was present in the first two post TIME0 intervals and further that this effect was dependent on water temperature with higher temperatures increasing the effect, i.e., resulting in a further decrease in the activity of recaptured fish (fig. 3). No significant effect of TL was observed.

Table 3**.** Results of GLM analysis of the effects of RECAP, TL and TEMP on the relative changes in activity. Activity in all four post TIME0 periods (DAY0-2, DAY1-3, DAY2-4 and DAY3-5) are compared to activity in the period before TIME0. Significant effects are indicated in bold.

	Type III SS	F	df	p-value
DAY0-2 (overall model)	1.129	3.833	4	**.008**
RECAP	.262	3.560	1	.065
TL	.002	.029	1	.864
TEMP	.020	.268	1	.607
RECAP*TEMP	.399	5.424	1	**.024**
DAY1-3 (overall model)	.901	2.694	4	**.041**
RECAP	.195	2.331	1	.133
TL	.065	.780	1	.381
TEMP	.000	.004	1	.947
RECAP*TEMP	.391	4.673	1	**.035**
DAY2-4 (overall model)	.688	2.041	4	.102
RECAP	.009	.109	1	.743
TL	.089	1.057	1	.309
TEMP	2.62E-5	.000	1	.986
RECAP*TEMP	.028	.336	1	.565
DAY3-5 (overall model)	.440	1.266	4	.295
RECAP	.021	.241	1	.626
TL	.094	1.085	1	.302
TEMP	.042	.482	1	.490
RECAP*TEMP	.001	.009	1	.923

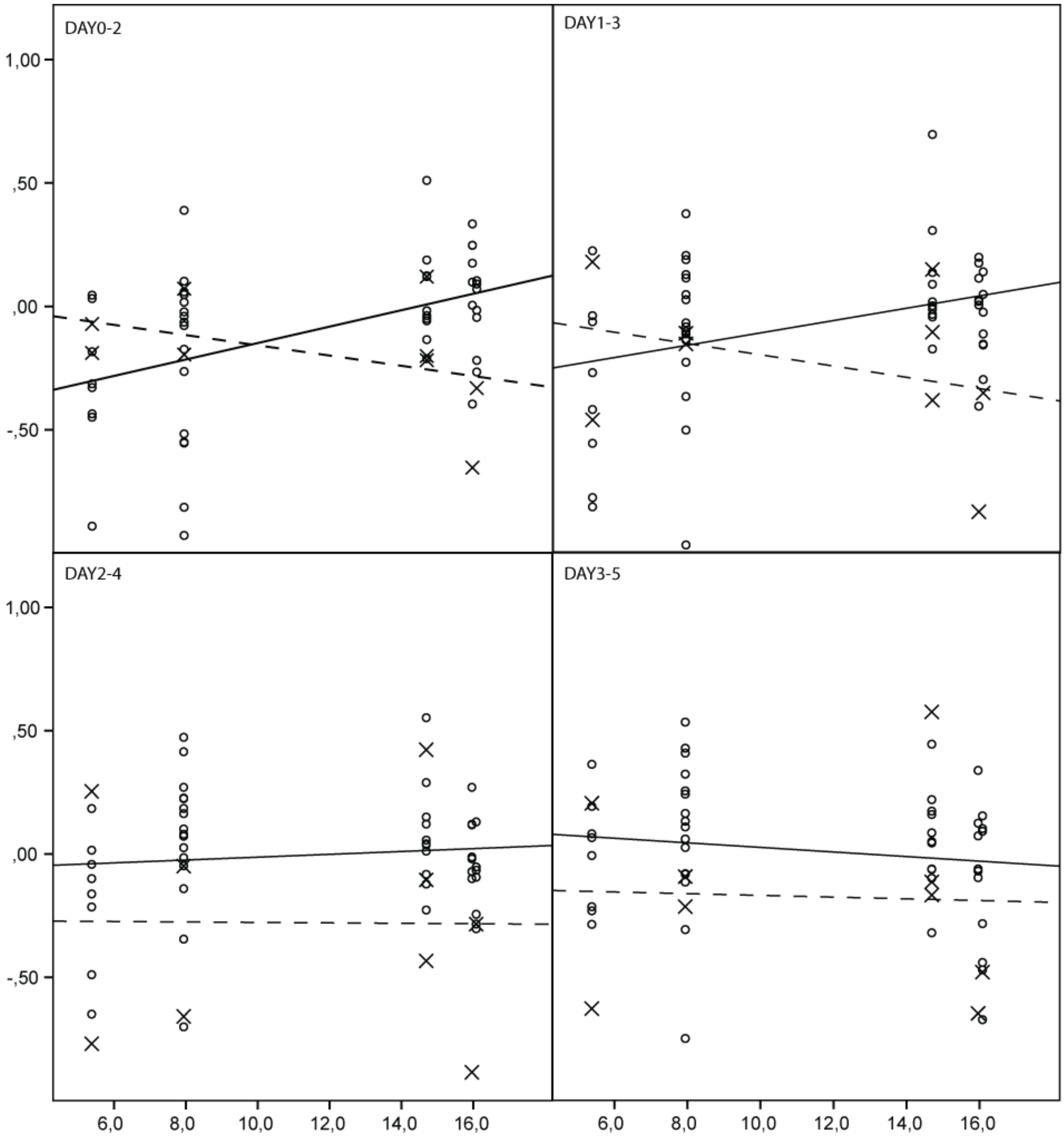

Fig. 3. Effect of water temperature on relative changes in activity in each of the four periods (DAY0-2, DAY1-3, DAY2-4 and DAY3-5) for recaptured (x) and control fish (o). The effect was significant for DAY0-2 and DAY1-3 as shown in table 3. Lines represent best linear fit of the data for the two groups (broken line: recaptured; solid line: control).

Discussion

The present study tested the effects of a handling protocol including catch-and-release on the volitional swimming activity of pike. This was done by contrasting activity before and after handling of recaptured fish compared with a control group. The results show that the handling protocol inflicted temporary changes in the relative activity of the treated pike compared to the control group and that this effect was temperature dependent.

From a management point of view the results are ambiguous. Indeed there was an effect of the recapturing, but it was transitory and could not be detected 48 hours post release. This suggests that recreational catch-and-release based fishing does not impart long term effects on the behaviour of the individual pike. However, there may be long term effects from catch-and-release that may not translate into changes in activity, e.g. physiological disturbances, though these have yet to be described (see Arlinghaus et al. (2009) for a discussion of catch-and-release induced physiological disturbances in pike).

In relation to future studies on pike behaviour, the results from the present study suggest that behavioural changes due to angling events of the focal fish are of a relative short duration. Moreover, since the handling protocol presented in the present study is based on angling, it is relevant to compare the present results with previous studies describing the effects of catch-and-release angling on activity levels of pike as e.g. Klefoth et al. (2008). Similar to the present study, they found that angled pike display reduced activity for a relative short period post release. Studies of catch-and-release in other species have also found reduced post release activity (Sundstrom & Gruber 2002; Gurshin & Szedlmayer 2004) and altered behaviour (Makinen et al. 2000; Thorstad et al. 2003). However, the strength and novelty of the present study is the inclusion of a control group of previously tagged fish that were not caught during the angling session in question. Assuming that tagged pike overall are not affected by long term post tagging stressors, this control group represents an estimate of the natural activity level of comparable conspecifics throughout the post angling periods. This approach increases the possibility of separating the effects of the actual catch-and-release treatment from uncontrollable whole system stimuli that could alter the activity levels of all individuals.

The continuous high resolution positional telemetry used in this study proved to be successful in getting detailed measures of the pike activity levels revealing large individual and temporal variation (fig. 2). This underlines the potential shortcomings of manual positional telemetry compared to automated telemetry regarding the resolution of data. If the present study had employed a manual tracking protocol (e.g. yielding ten positions per day), underestimation of the activity levels seems likely as discussed by Cooke et al. (2001) and Hanson et al. (2007) and it is possible that the observed effect of the handling regime would not have been detected.

The present study was done in a rather small lake effectively restricting the opportunities for the pike to perform long directional movements. Nevertheless, although all the tagged pike could traverse the entire lake within minutes, this was rarely observed (Baktoft, unpublished results), indicating that fine scale movements constituted a major part of their overall activity. Whether or not pike in larger lakes exhibits the same fine scale movements as observed in this study (in addition to longer displacements) is at present largely unknown, but it has been shown to be the case in smallmouth bass, *Micropterus dolomieu* Lacépède (Cooke et al. 2001). This warrants further studies, since the fine scale movements could represent a large and variable fraction of the activity not being observed using manual tracking, potentially clouding conclusions.

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Can metabolic properties explain variation in individual behaviour? Linking physiology and morphology with field behaviour

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Can metabolic or morphologic properties explain variation in individual behaviour? Linking physiology and morphology with field behaviour

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Abstract

Studies on animal behaviour often find considerable variation between individuals. This variation has historically been ignored as natural variation complicating an extrapolation from tagged individuals to population level. However, inter-individual variation in behaviour has recently been acknowledged as being an important ecological and evolutionary characteristic of wild populations but the mechanisms involved in maintaining such consistent differences are not fully understood. The present work explores potentially explanatory relations between inter-individual variation in behaviour, metabolism and morphology through an inter-disciplinary approach by combining laboratory protocols, a simple morphometric measure and high resolution telemetry on the same individuals. Sixteen European perch (*Perca fluviatilis*) were captured from a wild population, transferred to a laboratory and equipped with acoustic transmitters. Following a recovery period, morphometric and metabolic properties of each individual were determined. Finally, the fish were returned to their native environment, where an array of hydrophones allowed for automated continuous positioning of the fish. While data showed no correlation between metabolic properties and field activity, we identified links between individual morphology and behaviour. Thus, our analyses provide empirical field based data indicating that 1) individual metabolic properties are not strongly linked to volitional behaviour in free swimming fish, and 2) individual morphological differences affecting cost of transport are correlated with several behavioural measures.

Introduction

Unexplained inter-individual variation in the behaviour of otherwise similar conspecific individual has often been considered "noise", potentially obscuring a desired extrapolation from the observed animals to an overall population mean (Careau et al. 2008). However, in recent years, this residual variation has gained increased focus through the emergence of concepts such as behavioural syndromes, based on the recognition that individuals often display a degree of behavioural consistency across different situations (Sih et al. 2004; Sih et al. 2012). For instance, some individuals are consistently more responsive and active than others across different contexts like routine behaviour and novel object inspection. It has recently been suggested that such consistent individual differences in behaviour could be linked to metabolic properties (Biro & Stamps 2008; Careau et al. 2008; Biro & Stamps 2010).

Standard metabolic rate (SMR) is a measure of the basic maintenance requirements of resting post-absorptive unstressed animals below which physiological function is impaired (Priede 1985). At the other end of the aerobic scale, maximum metabolic rate (MMR) sets the upper limit of aerobic metabolism. Energy requirements of activities based on aerobic metabolism (e.g. routine swimming and gonadal development) are met within the SMR-MMR range, termed the scope for aerobic activity (SAA). Because these individual properties fundamentally set the limits within which the individual fish must survive, feed and reproduce, it is conceivable that they correlate with individual behaviour. Indeed, previous studies have found correlations between metabolic rates and several aspects of fish behaviour, e.g. positioning in schools (Killen et al. 2012b), vulnerability to angling (Redpath et al. 2010), behavioural dominance (Cutts et al. 2002), risk-taking (Killen et al. 2011) and migration propensity (Lans et al. 2011).

Interspecific differences in morphology of fish are known to reflect differences in swimming capabilities and general behaviour. For instance, the posterior positioning of dorsal and anal fin in pike (*Esox lucius*) reflects an adaptation to sprint based foraging (Craig 1996) while thunniform body shapes are optimal for cruising (Webb 1984). Likewise, intraspecific individual variations in body shape may affect the cost of transport in several species. For fish moving through water, overall body shape is a major determinant of resistive drag and thereby cost of transport. In hydrodynamic modelling of this, fish body form is typically simplified to e.g. a prolate spheroid and described by fineness ratio defined as length divided by maximum diameter. From such modelling, fineness ratios of approximately 5 to 8 have been shown to be most efficient (Blake 1983; Chung 2009). As such an optimum exist, it is conceivable that individual morphological differences will influence cost of transport and subsequently behaviour. Using a slightly modified version of fineness ratio, Ohlberger et al. (2006) found a direct relationship between swimming costs and fish morphology in carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*). Additionally, Boily & Magnan (2002) found that swimming costs were higher for stout than slender individuals of both brook char (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). Moreover, in the first study linking morphology and detailed behaviour of free-swimming fish, Hanson et al. (2007) found that body shape influenced both mean swimming speeds and travelled distance of nest guarding male largemouth bass (*Micropterus salmoides*). Although Boily & Magnan (2002) and Hanson et al. (2007) did not use fineness ratios, their findings are in line with Ohlberger et al. (2006) as the morphological measures used correlate with fineness ratio.

Most studies linking both functional morphology and metabolic properties to swimming performance and behaviour are performed in artificial laboratory settings. However, recent technological advents have enabled field studies on detailed fish behaviour (Lucas & Baras 2000; Cooke et al. 2005) thereby facilitating the inclusion of volitional behaviour of free-swimming animals in this research area (as in Hanson et al. 2007; Hasler et al. 2009a; Hasler et al. 2009b).

In the present study we test the hypothesis that individual behavioural variation is correlated with individual variation in physiological and/or morphological properties. To test this, we combined laboratory measurements of individual metabolic rates and morphology with individual behavioural parameters obtained from free-swimming perch. Individual standard and maximum metabolic rates and aerobic scope for activity from 16 perch were obtained using intermittent flow respirometry. Additionally, individual fineness ratio was determined as a measure of overall body shape. Subsequently, the same individuals were returned to their natal lake and following a re-habituation period of seven days, their undisturbed volitional behaviour was monitored for ten consecutive days using an acoustic telemetry array system which in effect turned the lake into a ‘monitored field aquarium’.

Materials and Methods

Fish

Twenty three perch (fork length (FL) range 141 – 197 mm) were captured in a small lake (Lake Gosmer; 55°55´42 N, 10°10´50 E; area approximately 1 ha; see Baktoft et al. (2012) for further info) using rod and reel and transferred to the laboratory. The perch were kept in square tanks (3 * 3 meters) using recirculated water kept at 16 (+- 1) degrees Celsius and fed daily with small live roach. Light regime was 12:12 (light:dark). Each perch was tagged with an acoustic transmitter (Lotek MAP 6_2, Burst interval 30 sec, 0.7 g in water) and a small PIT-tag (12 * 2.12 mm; 95 mg in air; LoligoSystems, Tjele, Denmark) to allow for rapid identification. Both tags were inserted through an incision in the body cavity following standard procedures as described in e.g. Jepsen et al. (2002). The fish were allowed a ten days recovery period before the onset on metabolic trials.

Metabolic properties

Respirometry was performed using automated intermittent closed respirometry as described in e.g. Svendsen et al. (2012). In short, four acrylic respirometer chambers were used concurrently allowing for simultaneous measurements on four fish. All chambers were submerged in ambient fully aerated water drawn from the recirculating system, i.e. the same water as the fish were held in. Ambient water temperature was kept at 16 (± 0.1) degree Celsius using thermostat controlled heater and cooler. Oxygen tension was continuously monitored at 1Hz using proprietary software (AutoResp, LoligoSystems, Tjele, Denmark) and subsequently post processed to yield oxygen consumption. Fish were isolated and unfed for 22 hours before trials.

MMR was determined using a customized version of the chase protocol described in Cutts et al. (2002) and previously adopted in e.g. Norin & Malte (2011) and Svendsen et al. (2012). Individual perch were transferred to a small tank and chased by hand until complete exhaustion as evidenced by the fish not reacting to being turned up-side-down and lifted partially out of the water. This typically took approximately five minutes. When exhausted, perch were immediately transferred to the respirometer and measuring of oxygen consumption commenced within 10 sec. The first measurement (always the highest for each individual) was assumed to represent MMR. Subsequently, the fish were left in the chambers for 20 hours until next morning. During this, the entire setup was shrouded in curtains to exclude visual disturbance of the perch. Additionally, each perch was visually shielded using vertical opaque acrylic screens. Pilot experiments showed that perch metabolic rate (MR) reached a stable low level shortly after the light went off. The mean value of the data acquired during the period of low and stable MR was used as a measure of SMR. To enable comparison between individuals, absolute MMR and SMR were converted to mass specific MR ($O2*kg^{-1}*h^{-1}$) using a scaling factor of 0.8 (Clarke & Johnston 1999) following this equation: Mass specific MR = $(1/\text{fish mass})^{0.8}$ * MR (Reidy et al. 2000). SAA was calculated as the absolute difference in mass specific MMR and SMR. Following each trial, all equipment was disassembled, disinfected and thoroughly rinsed. Pre- and post-trial runs showed a negligible background respiration from bacteria which therefore was disregarded in the analyses.

Morphology

Fork length, maximum body depth and breadth were measured to enable calculation of fineness ratio (FINE) as fork length divided by the square root of maximum depth*maximum breadth following Ohlberger et al. (2006). This definition of FINE was chosen as it makes more biological sense than the definition used in hydrodynamic modelling (length:maximum diameter ratio; Blake 1983) since a prolate spheroid is a poor descriptor of perch body form. FINE is a dimensionless measure of overall body shape in which low and high values indicate stout and slender individuals, respectively.

Behavioural measures

The perch were returned to their natal lake upon completion of the laboratory protocol. An acoustic positional telemetry system was used to obtain observations on volitional behaviour in Lake Gosmer. In short, the telemetry system enabled continuous monitoring of tagged fish with high temporal and spatial resolution by yielding time-stamped geographic coordinates (see Baktoft et al. 2012 for further details). From these data we calculated 1) daily individual activity as total moved distance in 24 hours (ACT; m * day^{-1}), 2) mean daily individual swimming speed (SPEED; m * s^{-1}) and 3) daily maximum individual swimming speed (VMAX; m * s^{-1}). Thus, one data point for all three measures was obtained for each individual each day. The first seven days were excluded to allow the perch to re-habituate to their natural environment. All behavioural measures were calculated from data obtained from the following 12 days during which the lake was left undisturbed.

ACT was calculated as the daily sum of Euclidian distances between consecutive positions. As described in Baktoft et al. (2012) there was a probabilistic component in the positioning system, potentially affecting the number of positions calculated for each fish each day (N_{obs}). To assess whether this influenced the ACT measure we examined the data for correlations between ACT and N_{obs}.

Point-to-point swimming speeds were calculated as the two-dimensional Euclidian distance between two consecutive points divided by delta time. To objectively and qualitatively optimize the data, only instances where the maximum obtainable temporal resolution, i.e. the transmitters burst interval (= 30 sec), were used. Furthermore, as the variable of interest was swimming speed, only periods where the individual perch were actually active were included. To objectively identify these periods we used a hidden Markov model with location and level of activity as hidden states (Pedersen et al. 2011) providing an activity metric, specifically the probability that the fish was active at a given time (P_{active}). Only observations with P_{active} >= 0.75 were included in calculation of SPEED. VMAX was defined as the daily individual maximum of the point-to-point swimming speeds where P_{active} >= 0.75.

Statistical analysis

Linear mixed effect models with random intercepts were employed following Zuur et al. (2009) and Pinheiro & Bates (2000) to analyse the data. By including FishID as a random effect, the models accounted for potential correlation between repeated measures on each individual. A series of models were fitted for each behavioural measure (ACT, SPEED and VMAX). In each model ACT, SPEED or VMAX was included as the response variable modelled by a common intercept (α), fork length (FL), one of the metabolic properties or the fineness ratio, FishID as a random effect (a) and residual noise (ε). For example: M_{SMR}: ACT ~ α + FL + SMR + a + ε (~ represents "modelled by"). The significance of each metabolic property and fineness ratio was tested by comparing the models to a reduced model, e.g. M_{RED}: ACT ~ α + FL + a + ε, thus excluding SMR in this example. Finally, M_{RED} was compared to a null model excluding FL, M_{NULL}: ACT ~ $\alpha + a + \varepsilon$. This approach was chosen over backwards elimination of a full model including all terms due to numerical problems caused by the relatively low sample size. All model comparisons were done using likelihood ratio tests as described in Zuur et al. (2009). Where appropriate, variance structures were included to accommodate residual heterogeneity (Zuur et al. 2009). Subsequent model validation plots showed no signs of violation of model assumptions. Fork length was chosen as a measure of body size as this is typically used in behavioural studies, although body mass is more commonly used in studies on metabolic rates. Fork length and body mass were highly correlated ($r = 0.97$) and could therefore not both be included in the statistical models. Pearson correlation coefficients were calculated using individual mean values. A prerequisite for the relevance of the present study was (at least some level of) consistency in the behavioural measures. This was assessed using the intraclass correlation coefficient (ICC) for each behavioural measure extracted from the mixed models following Zuur et al. (2009). ICC is a measure of within-individual correlation, i.e. the consistency of the observations made on each individual (Nespolo & Franco 2007). All statistical analyses were done in R version 2.12.1 (R Development Core Team 2010) using the nlme 3.1-97 package (Pinheiro et al. 2010).

Results

Of the 23 perch, three were consumed by tagged pike in the lake. The pike were tagged as part of another study in which it was estimated that more than 95% of pike over 40 cm were tagged (Baktoft, unpublished data). Additionally, four of the transmitters malfunctioned, leaving a total of 16 fish to be included in the analyses.

Intraclass correlation coefficients for the three behavioural measures were ACT: 0.99, SPEED: 0.14 and VMAX: 0.77. Thus, values of ACT and VMAX were highly consistent within individuals whereas values of SPEED were more variable.

The range of N_{obs} was 329 – 2433 equal to a position averagely every 262 – 35 sec. During the hours where the majority of perch activity took place (07:00 to 16:00) range of N_{obs} was 197 – 1057 equalling a position on average every 183 – 34 sec. There were no indications of correlation between ACT and N_{obs} (Pearson $r = 0.037$; Fig. 1). Therefore, Nobs was not considered in any subsequent analyses.

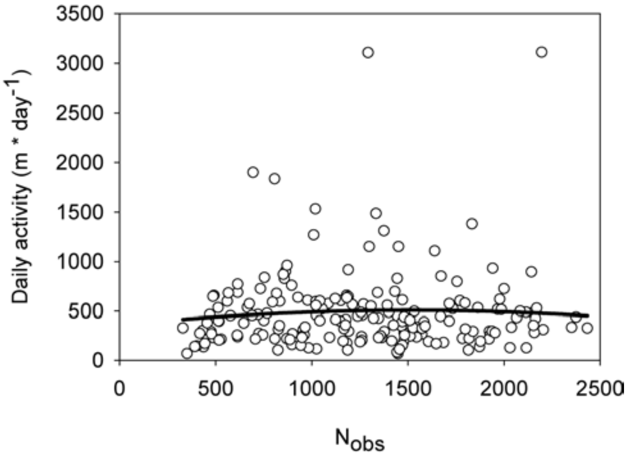

Fig. 1. Daily activity (ACT) plotted against N_{obs}. A quadratic curve fitted to the data is included to visualise that no correlation between ACT and N_{obs} is apparent.

There was no correlation between ACT and SPEED (Pearson r = -0.07; Fig. 2) indicating variation in the amount of time individual perch were registered as being active (i.e. P_{active} >= 0.75).

FINE and FL was not correlated (Pearson r = -0.14).

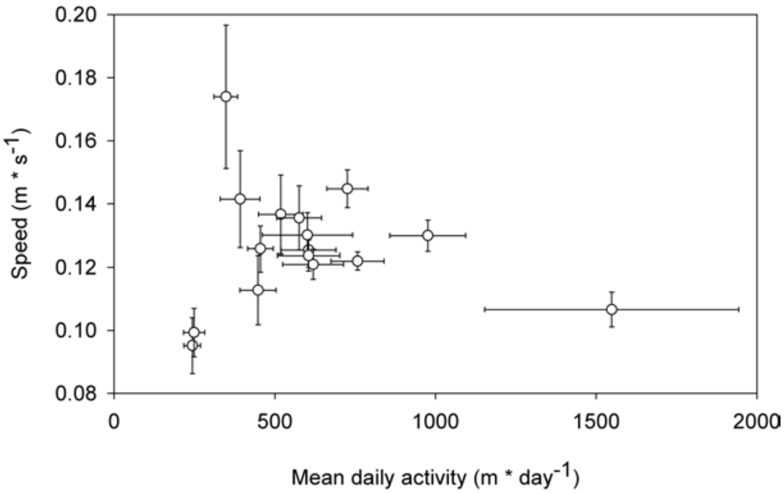

Fig. 2. Mean daily individual swimming speed as a function of mean daily individual activity (bars indicate s.e.m.).

Neither SMR, MMR, SAA nor FINE could explain a significant proportion of the residual variation in ACT when correcting for fork length (Table 1a). However, fork length did explain a significant amount of variation through a positive correlation (P = 0.0063; Fig. 3a).

FINE was positively correlated with the residual variation in SPEED when accounting for FL (P = 0.025; Fig. 3b). Neither of the metabolic properties explained a significant proportion of variation in SPEED (Table

1b). The isolated effect of fork length was not tested as fork length was included in the model testing for the effect of FINE.

Both FL and FINE explained a significant part of the variation in VMAX (P = 0.0064 and P = 0.0027, respectively; Table 1c; Fig. 3cd). Neither SMR, MMR nor SAA could explain a significant proportion of the variation in VMAX.

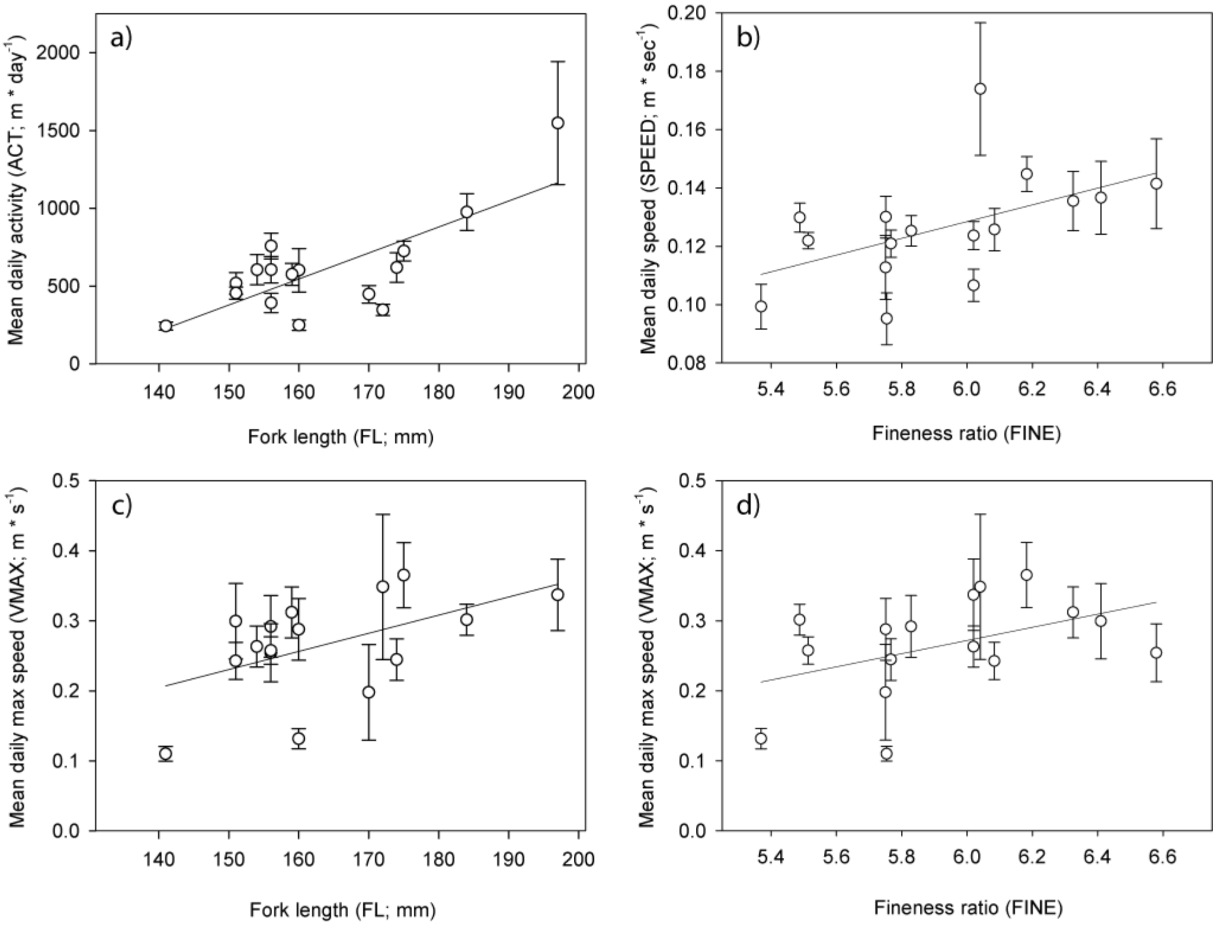

Fig. 3. Graphical representation of significant results from the mixed models in table 1. a) Mean daily activity (ACT) *vs.* fork length (FL) (P = 0.0063). b) Mean daily swimming speeds (SPEED) *vs.* fineness ratio (FINE) (P = 0.025). c) . Mean daily maximum swimming speed (VMAX) *vs.* fork length (FL) (P = 0.0027). d) Mean daily maximum swimming speed (VMAX) *vs.* fineness ratio (FINE) (P = 0.0064). All values are presented as individual means ± s.e.m. Solid lines represent the regression line from the corresponding mixed models.

Table 1. Results from mixed models testing the significance of MMR, SMR, SAA and FINE in explaining individual variation in three behavioural measures a) ACT, b) SPEED and c) VMAX. M_{NULL} was redundant in panel c as a more complex model was significant. Significant models are highlighted using bold face. Notice, FL was not significant in panel b model M_{FINE}, hence the final significant model was M_{RED2} (P=0.027).

a) ACT

Model		Test	Term tested	P
M_{MMR}	ACT ~ α + FL + MMR	M_{MMR} vs M_{Red}	MMR	0.45
M_{SMR}	ACT ~ α + FL + SMR	M_{SMR} vs M_{Red}	SMR	0.44
M_{SAA}	ACT ~ α + FL + SAA	M_{SAA} vs M_{Red}	SAA	0.95
M_{FINE}	ACT ~ α + FL + FINE	M_{FINE} vs M_{Red}	FINE	0.59
M_{Red}	**ACT ~ α + FL**	M_{Red} vs M_{Null}	FL	**0.0063**
M_{Null}	ACT ~ α	-	-	-

b) SPEED

Model		Test		P
M_{MMR}	SPEED ~ α + FL + MMR	M_{MMR} vs M_{Red}	MMR	0.62
M_{SMR}	SPEED ~ α + FL + SMR	M_{SMR} vs M_{Red}	SMR	0.33
M_{SAA}	SPEED ~ α + FL + SAA	M_{SAA} vs M_{Red}	SAA	0.72
M_{FINE}	SPEED ~ α + FL + FINE	M_{FINE} vs M_{Red}	FINE	0.025
M_{FINE}	SPEED ~ α + FL + FINE	M_{FINE} vs M_{Red2}	FL	0.69
M_{Red}	SPEED ~ α + FL	M_{RED} vs M_{NULL}	FL	0.85
M_{Red2}	**SPEED ~ α + FINE**	M_{RED2} vs M_{NULL}	FINE	**0.027**
M_{NULL}	SPEED ~ α	-	-	-

c) VMAX

Model		Test		P
M_{MMR}	VMAX ~ α + FL + MMR	M_{MMR} vs M_{Red}	MMR	0.72
M_{SMR}	VMAX ~ α + FL + SMR	M_{SMR} vs M_{Red}	SMR	0.77
M_{SAA}	VMAX ~ α + FL + SAA	M_{SAA} vs M_{Red}	SAA	0.73
M_{FINE}	**VMAX ~ α + FL + FINE**	M_{FINE} vs M_{Red}	FINE	**0.0064**
M_{FINE}	**VMAX ~ α + FL + FINE**	M_{FINE} vs M_{Red2}	FL	**0.0027**
M_{Red}	VMAX ~ α + FL	-	-	-
M_{Red2}	VMAX ~ α + FINE	-	-	-

Discussion

Metabolism

None of the examined metabolic properties (SMR, MMR and SAA) explained a significant amount of the variation in any of the three behavioural parameters (ACT, SPEED and VMAX). This is somewhat surprising since these properties represent the metabolic constraints within which the individual fish can perform aerobic fuelled activity including routine behaviour such as foraging and swimming (Priede 1985). SMR (or equivalents) are the most-studied aspect of vertebrate metabolism (Careau et al. 2008) and numerous studies have found positive correlations between SMR and behavioural parameters such as aggression, dominance and boldness in several taxa including fish (reviewed in Biro & Stamps 2010). However, empirical studies on the correlation between SMR and volitional movement activity in fish are scarce; a single study (Farwell & McLaughlin 2009) was identified in the review by Biro & Stamps (2010). In their study on recently emerged brook charr, Farwell & McLaughlin (2009) found no correlation between SMR and activity measured as time spent moving. Interestingly, Killen et al. (2012a) found positive correlation between routine metabolic rate (RMR) and activity measures under severe hypoxia but not in moderate hypoxia or normoxia. Moreover, Killen et al. (2012a) suggest that effects of RMR on behaviour might only be manifested during exposure to a stressor or that a stressor may amplify RMR-related differences in behaviour. Thus, although SMR correlates with some behavioural parameters, the link between SMR and activity in non-stressed conditions can be weak or non-existing as found in the present study.

Critical, maximum and optimal swimming speeds (U_{crit}, U_{max} and U_{opt} respectively; see Tudorache et al. 2008 for definitions) are measures of fish swimming performance obtained using laboratory protocols involving forced swimming (e.g. Claireaux et al. 2006; Tudorache et al. 2008). Although the mechanisms are not fully understood, these performance measures are linked to metabolic rates. For instance, Claireaux et al. (2006) present data suggesting that European sea bass (*Dicentrarchus labrax*) reach their maximum aerobic capacity at swimming speeds near U_{crit}. Furthermore, they found that oxygen consumption when swimming at U_{opt} represented a consistent percentage of MMR (Claireaux et al. 2006). Based on these findings a correlation between VMAX and MMR (and/or SAA) was expected but not found in the present study. Additionally, under the assumption often used in the literature that free ranging fish swim at or near U_{opt} during routine swimming (Claireaux et al. 2006), a correlation between SPEED and MMR and/or SAA was expected but not found in the present study. There are several possible explanations for this lack of expected correlations, including: 1) when determining U_{max} and U_{crit}, fish are typically forced to swim until exhaustion. Although these measures give insights in the maximum capacity of the fish, they may not be biologically relevant as is it currently unknown to what extent fish utilize their full aerobic potential in natural settings. 2) Similarly, the assumed relation between U_{opt} and spontaneous swimming speed of wild fish has yet to be confirmed (Claireaux et al. 2006). 3) The relatively long transmitter burst interval (30 sec) might integrate a period too long to solely reflect swimming activity (but see below).

In summary, the data showed no indications of the expected correlations between metabolic properties and behaviour. However, perch in the size range used will typically be feeding on either benthic invertebrates or pelagic prey fish (or a mix of these) (Hjelm et al. 2000) and may thus have constituted functionally different groups entailing different behaviours. There were some indications of differences in feeding strategy apparent from the morphological measure as discussed below. Therefore, metabolism-behaviour correlations may have been present within each functional group but this could not be tested in the present study as no information on individual feeding strategy was available.

Morphology

Individual fork length was positively correlated with both daily activity (ACT) and daily maximum swimming speed (VMAX) but not with mean daily swimming speed (SPEED). The positive correlation with ACT and VMAX were expected as larger fish generally utilize larger areas and are able to swim faster. Following this rationale, a correlation between FL and SPEED was also expected. These data could indicate that wild perch spend considerable time on activities with small scale movement in which case the burst interval of the used transmitters (30 sec) might have been too high to detect these movements. However, Zamora & Moreno-Amich (2002) found comparable swimming speed of perch obtained in the field (average 10.4 cm * s-1) albeit in larger fish (24 – 26 cm). Furthermore, Linlokken et al. (2010) found swimming speeds in laboratory experiments on perch foraging behaviour comparable to the present study when correcting for differences in fish length (ca. 5 – 7 cm * s^{-}1 @ mean TL = 11.8 cm (total length) in Linlokken et al. 2010 *vs.* ca. 10 – 13 cm * s^{-1} @ mean FL = 16.4 cm in the present study). This indicates that measured SPEED in the present study is within a credible range.

Fineness ratio explained significant proportions of the variation in both SPEED and VMAX. Fineness ratio is a dimensionless measure of body slenderness in which higher values indicate more slender bodies. Previous studies have shown that higher fineness ratios (up to a given threshold) generally are associated with lower swimming costs (Boily & Magnan 2002; Ohlberger et al. 2006). Additionally, it is known that morphological polymorphism in perch is associated with differences in foraging efficiency and search velocity in pelagic versus littoral habitats (Svanback & Eklöv 2004). The only previous field study linking detailed fish behaviour with morphological characters found correlations between a composite morphometric measure comparable to FINE and mean speed and mean daily distance in largemouth bass (Hanson et al. 2007). The present study adds empirical field data emphasising the biological relevance of individual morphological differences. Although the prospect of a pure physical explanation (i.e. the hydrodynamic effects of fineness affecting swimming costs) of parts of the individual variability is alluring, this correlation most probably entails some biological components as well. For example, fineness ratio could be related to nutritional status as ‘fatter’ fish of a given length will have a lower fineness ratio. Moreover, we used perch whose morphology is known to be highly plastic and to correlate with both habitat structure and feeding mode (Olsson & Eklöv 2005). Generally, deep bodied and thus more manoeuvrable perch are associated with the benthic niche, whereas slender perch are associated with pelagic feeding (Hjelm et al. 2000; Svanback & Eklöv 2004). However, regardless of the causal mechanism driving the correlations between fineness and behaviour, it is interesting that a simple metric can explain significant amount of inter-individual behavioural variation.

The present study relies on several crucial assumptions. First of all, we assumed that the measured metabolic rates were repeatable and temporally consistent. This could not be verified due to time restrictions set by the transmitter battery life, but previous studies suggest that this was a valid assumption (Nespolo & Franco 2007; Maciak & Konarzewski 2010; Norin & Malte 2011). Analogous, some level of consistency in the behavioural measures was a prerequisite for the relevance of the present study. This criterion was met for both ACT and VMAX as suggested by their respective ICC values, whereas the intra-individual consistency of SPEED was considerably smaller. Additionally, we assumed that the behavioural parameters obtained using the telemetry system truthfully reflected fish behaviour. The validity of the system has previously been assessed by towing transmitters to mimic swimming fish. These tracks showed very good concordance with true tracks obtained using DGPS (Baktoft et al, in prep). The estimation accuracy of behavioural parameters (especially SPEED and VMAX) might have been influenced by the relatively long burst intervals (30 sec). The selected BI was chosen as a compromise between battery life expectancy (i.e. longevity of the study period) and transmitter size/weight. Finally, it should be noted that the results in the present study are based on a relatively low sample size, partly due to misfortunate happenings, i.e. transmitter failure and predation. Therefore, care should be taken when interpreting the results. However, even when correcting for pseudo-replication by using a mixed model approach, several of the findings were highly significant adding credibility to the results.

In summary, the present study suggests that although the metabolic properties define the aerobic capacities of fish, a direct link to volitional routine behaviour is missing. In contrast, we found several indications that fish size and morphology correlates with fish activity, suggesting a stronger link between these factors.

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