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Att mäta puls och furageringsbeteende hos fisk via elektroniska sensormärken

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Examensarbete i ämnet biologi
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Abstract

Modern electronic sensor tags (BioLoggers) can collect high resolution data on movement and physiology of fish. These tools make it possible to remotely monitor physiological stress responses as well as complex behaviours, such as feeding and hunting, in free swimming wild fish, data that often is difficult to collect by other means. Quantification of stress and feeding behaviour is valuable in behavioural ecology and fish conservation, and are also important in fish welfare (e.g. in hatchery environments and in fisheries). This study had two main goals: 1) Evaluating the use and function of a newly developed cord-less heart rate bio-logger to measure stress in hatchery brown trout (*Salmo trutta*), and 2) Evaluating the use of a high resolution 3D accelerometer bio-logger to remotely capture different foraging behaviours in Eurasian perch (*Perca fluviatilis*). Bio-loggers are often inserted surgically in the fish, and in study 1 the first aim was to evaluate if the placement of the bio-logger inside the fish affects the quality of the data produced. Results showed that placement close to the heart produced significantly higher quality of data compared to when the logger was placed in the belly of the fish. The direction of the logger with regards to the electrodes did not impact the quality of the data. Based on this result, a protocol for the surgery and placement of cord-less HRT-sensors in trout was developed. To evaluate the ability of the bio-logger to capture change in heart rate as a function of stress, hatchery brown trout was tagged with heart rate sensors and exposed with a GABAergic anxiolytic drug known to reduce stress in fish. The fish was then exposed to handling stress, and heart rate in exposed fish was compared to control fish that was not exposed to the anxiolytic drug. The result showed that the control fish had significantly higher heart rate during handling periods compared to non-handling periods, but that such difference could not be seen in the drug exposed fish, hence indicating that the new cord-less heart rate logger is capable of quantifying induced stress and stress relief in fish. In study 2, two different feeding behaviours in perch, feeding on small insects and feeding on small fish, were carefully studied in aquaria. The two behaviours were then manually simulated using a euthanized perch tagged with 3D accelerometer bio-logger. The data collected by the accelerometer was then analyzed using a machine learning approach, with the aim to be able to distinguish between the two feeding behaviours using only the acceleration profiles. The result showed that it is possible to distinguish between feeding behaviours in perch with high accuracy (99 %) using acceleration bio-loggers. The results are discussed based on the potential usability of heart rate and accelerometer bio-loggers in animal welfare and ecological research.

Introduction

How often does a fish eat? What does it eat? How does fish react to stress in its environment? These questions are important to find answers to when working with welfare questions or when trying to understand adaptation and evolutionary processes in fish (Cooke *et al.*, 2016). However, it's often difficult to collect such data from animals in aquatic environments. One fairly recent technical development that allows the collection of behavioural and physiological data is electronic sensor tags. Today electronic tags are used in conservation work to for example quantify disturbance, estimate energy expenditure and also to understand consequences of habitat selection and animal movement (Hussey *et al.*, 2015; Wilson *et al.*, 2012). Electronic sensor tags can measure depth, temperature, fluorescence, conductivity (Hussey *et al.*, 2015), heart rate, swim speed, stroke frequency, heat flux, muscle oxygen, acceleration and many other parameters. Some of these parameters can be used to quantify feeding events, predator-prey interactions, also social behaviour as well as quantifying stress (Ponganis, 2007). Today, the most commonly studied parameters in fish are heart rate, swim speed and body temperature (Ponganis, 2007).

Some attempts to use electronic tagging tools in fish science were done already in 1950s, but it was only during the 1990s that commercial tags became available (Cooke *et al.*, 2016). Today a big assortment of tags for fish is available from commercial manufacturers such as Maritime bioLoggers, Gulf Coast Data Concepts, Vemco-Amarix, Thelma BioTel and Advanced Telemetry Systems (Cooke *et al.*, 2016).

A bio-logger refers to an electronic sensor tag that you attach to an animal to provide data about behaviour, physiology, movement or the environment it lives in. Modern bio-loggers can collect large amounts of data at a high frequency (i.e. sampling rate) (Fehlmann & King, 2016). The data is saved in an on-board memory that must be retrieved to get access to the data that has been collected (Fehlmann & King, 2016). In aquatic science, transmitting bio-loggers (i.e. accessing data without retrieving the logger) are rare, as the amount of data collected by bio-loggers usually are very large and hence difficult to transfer under water due to the narrow bandwidth of current underwater communication technology (Hellström *et al.*, 2016a).

In the beginning, bio-loggers was only used on big marine species such as seals and wales. The big battery that was needed made the size of the logger too big for smaller animals. With the development of smaller and more efficient batteries, bio-loggers got smaller in overall size, making them more suitable to use on smaller animals. These days the bio-loggers can be applied to a big range of species both aquatic and terrestrial, such as birds, reptiles, mammals, insects and fish (Ropert-Coudert *et al.*, 2012).

Welfare questions answered with heart frequency loggers

There is an increasing demand of food for a growing human population (Crute & Muir, 2011). To meet this demand, food production must become higher, and it's important that this increase is done with a sustainable and efficient use of resources (Crute & Muir, 2011). Aquaculture are rapidly growing and becoming a big and important sector for both regional and global economies (Cooke *et al.*, 2004). Recently, the uses of bio-loggers in animal welfare have gained traction, especially in earlier neglected species such as fish (Relić Renata *et al.*, 2010). The fact that fish can feel pain and suffering (Relić Renata *et al.*, 2010) makes fish welfare an important subject, especially in an aquaculture context where living fish are treated industrially, and exposed to potentially stressful handling (Relić

Renata *et al.*, 2010). In fish, the reaction and impact of stressors are similar to mammals, and the assessment of welfare for farmed fish are built on the same principles as for terrestrial farm animals (Relić Renata *et al.*, 2010). Stressors in fish farms can be handling, inadequate stocking densities, bad water quality and food that don't meet the physiological needs of a species. A long-term exposure to these stressors affect fish health and productivity negatively (Relić Renata *et al.*, 2010). Hence, when working with fish welfare, it is important to acquire reliable measurable indicators of stress (Relić Renata *et al.*, 2010), and this has traditionally been difficult to do. Bio-logging, however, is a promising technology to acquire such data in fish.

Bio-loggers that measure heart rate are effective for monitoring physiological responses to a variety of conditions in fish and other animals. Heart rate sensors have been used on for example humans to evaluate energy expenditure (Silva *et al.*, 2014), laboratory mice in pharmacological, cardiovascular and toxicological studies (Cesarovic *et al.*, 2011) and also in hibernation studies in black bear (Laske *et al.*, 2014). Heart rate is often related to locomotion, and there may hence be a relationship between heart rate and swimming activity in fish (Cooke *et al.*, 2002).

Heart rate has also been used as a measurement of stress (Clark *et al.*, 2010; Cooke *et al.*, 2002). This is possible because cardiovascular functions change as a response to stress (Barton, 2002). Heart rate can hence be used to answer several welfare questions related to stress, such as how different densities of fish in aquaculture affects stress (Cooke *et al.*, 2000) or how handling time affects the physiological and behavioural recovery time of the fish (Raby *et al.*, 2015). Heart rate loggers can also be used in pharmacological studies to measure effects of pharmaceuticals in fish (Hellström *et al.*, 2016b; Makiguchi *et al.*, 2009). There is hence a great potential for this type of technology to increase our understanding of how to minimize stress and improve the animal welfare conditions in aquaculture (Cooke *et al.*, 2000).

Traditional heart rate ECG bio-loggers are requiring invasive surgery to be able to collect high quality data (Baras & Lagardère, 1995). Functional logging and placement of these tags is based on bio-electrodes which are inserted into the muscles near the pericardium through a system of cords (Baras & Lagardère, 1995). Apart from being a difficult surgical operation, the cord-based bio-electrodes are invasive and may cause increased stress to the fish (Relić Renata *et al.*, 2010). Recently new cordless heart rate bio-loggers, which are easier to implant, were introduced on the market (Star-Oddi, 2017a). This means easier tag-deployment and surgery, and hence less stress for the fish. However, so far no study has evaluated the use and function of these new cord-less heart-rate bio-loggers.

Basic ecology and acceleration bio-loggers

Bio-loggers measuring acceleration are becoming frequently used when collecting information about animal behaviour and movement ecology (Cooke, 2008). Accelerometers are devices that can be attached to animals to detect overall activity levels, body orientation and specific behavioural patterns (Soltis *et al.*, 2012). Bio-loggers measuring acceleration have been used on terrestrial, flying and aquatic species (Bidder *et al.*, 2014), and have today been applied to more than 120 species (Brown *et al.*, 2013).

A very promising area of use for acceleration bio-loggers is the determination of acceleration profiles of specific behaviours in animals. These profiles can then be used to

retrospectively detect these specific behaviours in free-ranging wild animals tagged (and recaptured) with accelerometers (Resheff *et al.*, 2014; Whitney *et al.*, 2010).

The use of accelerometers to identify behavioural modes has been pioneered in terrestrial animals, predominately birds and mammals (Bom *et al.*, 2014; Nathan *et al.*, 2012). An inherent problem of acceleration data measured using high-frequency bio-loggers is the large size, often ranging from tens of thousands to millions of data points (measurements), making analyses challenging. With modern powerful computers, acceleration based classification and identification of behavioural modes can be done automatically using supervised machine learning algorithms or other analytical classification methods. Often, distinctly dissimilar behaviours are easily distinguished using such methods, whereas behaviours that are more similar can be confused with each other (Soltis *et al.*, 2012). Acceleration data loggers has been used on African elephants (*Loxodonta africana)* to record and distinguish between behaviours like bathing, feeding and walking (Soltis *et al.*, 2012). Acceleration data have been used on lemurs (*Lemur catta*) to measure the distance the animal travels, as well as identifying leaping behaviour (Sellers & Crompton, 2004). The behavioural mode can be combined with GPS-position of the animals to examine interactions between ecological, behavioural and biomechanical aspects of movements (Nathan *et al.*, 2012), providing a potentially very powerful way of getting insight into what affects animal behaviour. Acceleration data has also been used in human health research to classify patients behavioural modes such as walking, running and sitting (Nathan *et al.*, 2012).

Although there is a big potential of accelerometers to remotely collect data on animal behaviour, the use of accelerometers to identify behaviours in fish is still in its infancy. Behaviours of wild aquatic animals are often very difficult to study, especially without disturbing them. This makes accelerometers a very interesting tool, and acceleration data has already been used to classify mating behaviour and other behaviour in free-living sharks (Whitney *et al.*, 2010), and to determine specific feeding behaviour in toadfish (de Almeida *et al.*, 2013). However, few studies have evaluated the use of a machine learning framework to identify foraging behaviours in fish based on acceleration data.

Aim of study

This study has two aims. The first aim is to evaluate the use of a newly developed cord-less heart rate bio-logger, from the company Star-Oddi (DST micro-HRT logger), to measure stress in hatchery reared brown trout (*Salmo trutta*). This was conducted by: (1) studying the best placement of the DST micro-HRT logger in the fish to give the highest quality measurements of the heart frequency and (2) investigating the ability of the bio-logger to capture change in heart rate following handling stress in farmed fish.

The second aim is to evaluate the use of a high resolution 3D accelerometer bio-logger to remotely capture different foraging behaviours in Eurasian perch (*Perca fluviatilis*). Here, we investigated whether it was possible to distinguish between the two different feeding behaviours (eating insects and eating fish) based solely on the acceleration profiles of the behaviours, using supervised machine learning analytics.

The different experiments will be presented with regards to the two different aims where the first aim are presented under headline “Welfare questions answered with heart frequency loggers” and the second aim under the headline “Basic ecology and acceleration bio-loggers”.

Materials and methods

Welfare questions answered with heart frequency loggers

Star-Oddi DST micro-HRT bio-logger

The newly developed Star-Oddi DST micro-HRT bio-logger records heart rate via a leadless single channel ECG sensor that consists of three electrodes. The bio-electrodes are part of the loggers housing material (Fig. 1) which makes the logger easy to implant (i.e. no cords needed as with traditional HRT-loggers (see example of a traditional hrt-biologger in Fig. 2). The DST micro-HRT bio-loggers have a dimension of 8.3mm x 25.4mm and weighs 3.3 g. The logger measures long term heart rate and core temperature simultaneously.

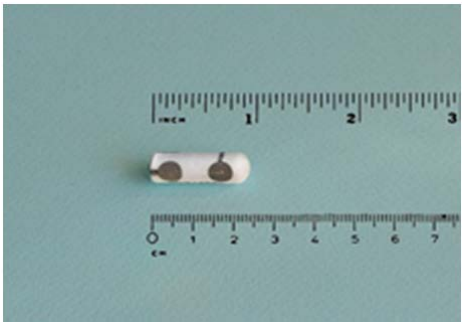

Fig. 1. DST micro-HRT logger from Star-Oddi (Star-Oddi, 2017a).

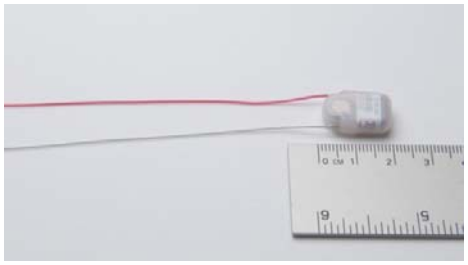

Fig. 2. Example of a traditional heart rate bio-logger with the electrodes inserted into or close to the heart muscle (DSI's PhysioTel® ETA-F10 transmitter, (Datasci, 2017)).

Each heart rate value is obtained from the ECG profile, using the so called RR-interval, i.e. the distance in time between the parts of the ECG-curve that capture the peak deflection of the heart during one contraction (R-wave), from a period of 600 samples (Fig 3). The sampling frequency, or Hertz, can bet set by the user, and range between 100-800 Hz. So if sampling with a frequency of 100 Hz, the logger will sample over a 6 second period to generate 600 samples. For visualization of the full ECG, all measurement points (PQRS) collected by the logger can be saved in a separate file, a so called buffer file.

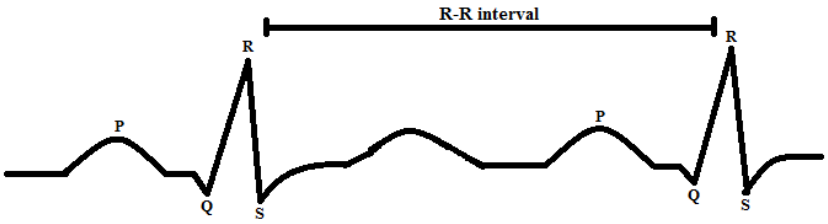

Fig. 3. The complete PQRS profile of a heartbeat and the RR-interval (Luz *et al.*, 2016). The RR-interval functions by storing the interval between each heartbeat or storing the number of heartbeat per a certain time period (Ponganis, 2007).

Each heart rate value (beat per minute) recorded by the logger is graded with a verification quality index (QI), which gives an indication of the quality (i.e. the accuracy) of the measurements. The QI range from 0-3, where 0 represent great quality, and 3 poor. The basis for how the quality index is calculated is not known to the author, despite several attempts to have Star-Oddi explain it. The software supporting the logger is called Mercury. In the software the user can set factors like start time, date and the sampling interval (Star-Oddi, 2017a), as well as get access to recorded data.

Placement study

In this study, two DST micro-HRT loggers were used simultaneously. The loggers were set to have a sampling frequency of 125 Hz, sampling for 5 seconds every 15 seconds. It was also set to collect a buffer data file at every measurement, i.e. the whole ECG signal was saved. The loggers then sampled continuously until the memory became full (approximately 50 minutes).

Trout used in this experiment originated from a fish farm in Norrfors near Umeå, Sweden. Two year old trout was collected from the hatchery stock, and transported to 600 L holding tanks in a lab at the hatchery. As the experiment started the trout were individually anaesthetized with ms222. When the fish showed loss of equilibrium and no response to manual stimulation it was considered fully anaesthetized. It was then moved to a surgical box where it was fixated on its back using water-soaked foam-cushions. The gills were submerged under water during surgery, and a pump was pumping aerated water thru the gills to ensure that the trout obtained sufficient oxygen during surgery. An approximately three cm long incision was made from between the anal fins up to between the pelvic fins. This opened up the belly of the fish, which created space to place the logger at different positions inside the fish.

The loggers have three electrodes; two electrodes are placed at one end and one electrode at the other. To study the best location of the tag inside the fish for receiving good quality signals, the loggers were placed at two different positions inside the trout and turned in one of two different directions. This generated four different placements of the tag inside the fish: (1) near the heart with the two electrodes down towards the dorsal fin, (2) near the heart with the two electrodes up towards the pelvic fins, (3) in the belly with the two electrodes down towards the dorsal fin and (4) in the belly with the two electrodes up towards the pelvic fins (Fig. 4).

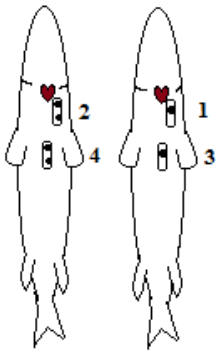

Fig. 4. Placements of DST micro-HRT loggers during placement study. The angle of the fish represents the underside of the trout. 1= near the heart with the two electrodes down towards the dorsal fin, 2= near the heart with the two electrodes up towards the pelvic fins, 3= in the belly with the two electrodes down towards the dorsal fin, 4= and in the belly with the two electrodes up towards the pelvic fins.

When placing the logger near the heart it was gently pushed close to the pericardium and then rotated into in the right position. In the belly the logger was placed beside the intestine.

The experiment started by inserting one logger near the heart and the other in the belly, both with the two electrodes down toward the dorsal fin. After five minutes the loggers were rotated so that the two electrodes were directed up towards the pelvic fins. After five minutes the loggers changed position. Then the same procedures as before with changes of the directions of the electrodes were repeated. Each fish was studied for 20 minutes (each tag at each position in the fish). After the operation all trout was euthanized by pithing through the brain stem. In the surgery experiment six trout were used.

Based on these measurements three different quality variables were derived: (1) QI for the sampled data in each placement, (2) variance of heart rate for each QI and (3) difference in QI and heart rate between loggers when placed at the same position. These variables were then used in the subsequent testing of the effect of placement on heart rate data quality. QI data was aggregated into two categories, where QI< 3 was categorized as good quality observations, and QI=3 categorized as bad quality observations.

The effect of tag placement on QI was tested using a generalized linear mixed model with binomial errors, with TagID and FishID kept as random effects in a nested structure (TagID/FishID). Effect of placement was evaluated in two steps, first testing the difference in QI between placement close to the heart vs. in belly, and second, testing the effect of tag-direction.

Measuring stress response

Sequence of events

The purpose of this experiment was to investigate whether the DST micro-HRT loggers were able to detect changes in heart rate following handling stress and drug exposure in hatchery reared brown trout. 20 two year old trout were transferred from the hatchery stock to the hatchery lab and kept in 100 L holding tanks. Two of the trout were implanted with a

DST micro-HRT logger set to sample for 5 seconds every 15 second during one hour a day (between 11:00 and 12:00 AM) at a frequency of 125 Hz. The loggers were placed close to the pericardium, following a surgical procedure similar to what has been described above for the placement study. A one cm incision was made in between the pelvic fins, through which the logger were inserted and pushed forward toward the pericardium. The incision was closed with two stiches, using non-degradable sutures. Each fish was fully anaesthetized by ms222 during surgery. The trout was then moved back to the tank for recovery from anesthesia. The procedure took around two minutes for each fish.

After recovery, the trout were separated and moved to two aquariums containing 9 untagged and 1 tagged trout in each. The non-tagged fish in each aquarium created a social environment for the focal trout, and hence was intended to reduce the stress from residing in the new environment. The water in one aquaria was exposed to a GABAergic anxiolytic drug, Oxazepam. Oxazepam is a commonly used agent in anxiolytic pharmaceuticals. It is used to decrease anxiety in humans, but has also shown to reduce anxiety in fish by affecting the GABAergic system in the brain (Brodin *et al.*, 2014), a finding that has caused concern among conservationists as Oxazepam is found in surface waters around the world. The other aquarium contained clean water and hence functioned as a control. To keep the concentration of Oxazepam at stable levels, the aquaria had no flow-through and instead oxygen pumps were used to maintain a healthy oxygen supply. In this study, exposure concentration of Oxazepam was set to 100 µg/l, which is approximately 100 times higher than the highest concentration reported from the environment, typically ranging from ng 1^{-1} to low µg 1^{-1}(Brodin *et al.*, 2014).

Handling stress

The experimental period started with a post-surgery rehabilitation time of six days. During these six days the tagged individual in the experiment group reached steady-state with the pharmaceutical in the water (i.e. uptake and elimination of the pharmaceutical was identical). No heart rate measures were taken by the loggers before steady-state was reached. After six days, the fish were handled with a ring net for 1h a day, between 11:00 and 12:00, and the loggers were set to measure heart rate. Fish from one aquarium was handled at a time and handling was done by capturing the trout from the aquarium by using a ring net, then holding them in the net above the water for ten seconds. After that, the fish were released into a different water container. When all ten fish had been removed from the aquarium they were captured and held in the net for ten seconds again after which they were released back in to the aquarium. This sequence of events was then repeated with the trout in the other aquarium. The sequence was then alternated between the groups which resulted in a handling sequence of one fish group every tenth minutes. This handling routine mimics the handling a trout can be exposed to in a hatchery, although this probably was more intense as the fish was handled multiple times during one hour, something that is unlikely in a hatchery setting.

In between the handling periods, the aquaria were partly covered with blankets to reduce the stress imposed on the trout by external stimuli, and the trout was fed with pellets from the fish farm. The handling treatments were repeated during four consecutive days (1h/day) so each group was handled a total of 16 times. The loggers then continued to record for four more days (1h/day, between 11:00-12:00). During these four days, the fish were left undisturbed. This makes a total of eight sampling occasions by the logger for both the exposed and unexposed individual.

Data analysis

Difference between exposed and control fish in the effect of handling on heart rate was tested using a general linear mixed model, with heart rate as the response variable, handling as a two-level fixed effect (handling/no handling) and date as a random factor. Separate models were used for exposed and control fish. The reason why not exposed and control fish were contrasted in the same model (i.e. via an interaction effect) was that only two loggers were used and only two fish, and hence that there was a risk for a consistent bias in heart rate between the tags because of uneven factory calibration, or that the two fish may have had consistently different resting pulse. Only detections with a QI below 3 was used in the analysis. The data was processed in both Mercury and Excel. The statistical analysis were made in R. R is a programming language that can be used for data visualization and statistical calculations.

Basic ecology and acceleration bio-loggers

Overall aim: evaluating the use of Star-Oddi DST tilt loggers to remotely capture two different feeding behaviours in perch; eating insects and eating fish.

Overall experimental setup: The two different feeding behaviours of perch were studied in detail in aquaria by feeding the perch insects and prey fish. The behaviours were then imitated manually using a euthanized perch tagged with a DST-tilt logger. An analytical classification method was then used to try to distinguish between behaviours using only acceleration data collected by the DST-tilt logger as input.

The Star-Oddi DST tilt logger (Fig. 5) can be used to monitor fish movement underwater or to evaluate the function of gear that is utilized underwater (i.e. nets, lines, trawl doors). It measures acceleration and tilt, both variables measured in relation to earth's gravity. It also measure depth (m) and temperature (°C). The logger stores the data in an internal memory with a timestamp for each measurement. The software SeaStar (Star-Oddi) is used both to configure and to collect the data from the logger. Starting time, start date, and up to seven different sampling intervals for the same sampling sequence can be set when configuring a new sampling interval. A burst function enables you to take samples at high frequency (5 sample/sec). The logger can be reused as long as the battery lasts (Star-Oddi, 2017b).

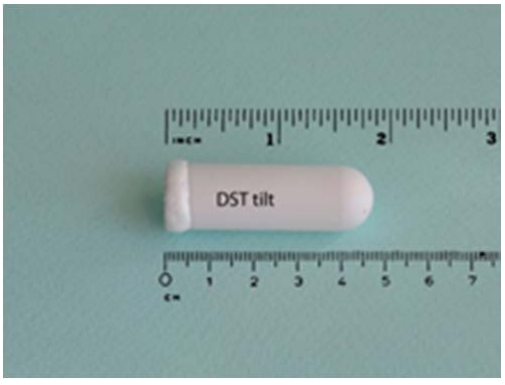

Fig. 5. The DST tilt logger from Star-Oddi (Star-Oddi, 2017b).

The DST-tilt logger measure acceleration at very high rates in three axes, the heave (vertical), sway (horizontal) and surge (forward) axes (Fig. 6) (Cooke *et al.*, 2016; de Almeida *et al.*, 2013).

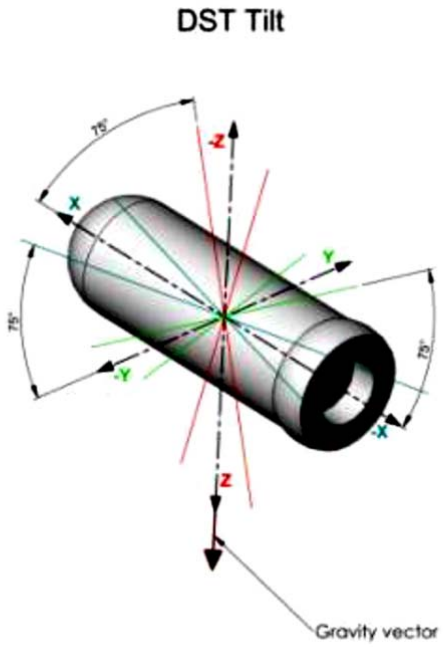

Fig. 6. Shows the direction of tri-axis Z,Y and X (Star-Oddi, 2017b).

By using the software SeaStar the logger was set to sample acceleration and temperature once every second, but by using the burst function it can take five samples every second. The total measurement time was two hours.

Sequence of events

Three perch (approximately 20 cm) were captured in Umeälven near Umeå in northern Sweden, and transferred to a nearby lab at the hatchery in Norrfors. The perch were maintained in an indoor tank and fed with juvenile salmonids from the hatchery until the study started. Eight days after arrival, the fish were separated and put in three different aquaria. The aquaria were equipped with a system that created a continuous water flow. All but one of the aquarium walls were covered with white colored plastic so the fish would be clearly visible when observed. The light was set to optimize the visibility of the fish and the perch were acclimatized to the new environment for seven days.

To observe how perch behaved when eating insects, dragonfly nymphs, caddisfly larva and stonefly nymphs were collected in a small pond and fed to the perch (Fig. 7). To observe how perch behaved when eating fish, small crucian carps (*Carassius carassius*) was collected from the same pond as the insects, and fed to the perch. The average length of the crucian carps were 6 cm.

Fig. 7. Insect and small crucian carps were collected in a small pond at Röbäcksdalen, Umeå.

A Sony digital HD video camera recorder model HDR-XR260VE was used to record the perch during feeding. The perch was recorded individually while first being fed insects and later crucian carps. Each perch were fed and recorded once a day during four days. In total 22 attacks were recorded, 14 were attacks on crucian carps and eight on insects. After the recordings, the perch were released close to the place where they were captured. During the periods between the recordings the aquaria where partly covered with nets and blankets to avoid unnecessary stress for the fish.

The recorded foraging behaviour was thoroughly studied so that the behaviour could be recreated manually. The logger was surgically implanted into a euthanized perch of approximately 20 cm. Two sutures were used to lock the logger at a fixed position inside the perch (Fig. 8). This was done to prevent the logger from rotating inside the fish.

Fig. 8. Two sutures were secured at the front of the logger.

The fish was then held in a water filled aquaria and moved by hand to simulate the recorded behaviour (Fig. 9). 104 fish attacks on crucian carps and 62 attacks on insects were simulated. All movements were recorded by two web cameras. Later these recordings were used, along with the acceleration measurements taken by the logger, to clearly set the start and stop time for specific behaviours on the acceleration data. In this part of the study only one perch was used.

Fig. 9. A euthanized perch used to mimic perch foraging movements by moving it by hand.

Data analysis

The acceleration data captured by the DST-tilt logger was downloaded from the logger using SeaStar. This data was then fed into the software AcceleRater (Resheff *et al.*, 2014), a python-based web application that can be used for automatic classification and identification of behaviours from accelerometer data using a machine learning approach (Bidder *et al.*, 2014). A set of different classification models are used by the software (e.g. various ordinal regression methods) to try automatically classify behavioural events based on the consecutive observations of the heave, sway and surge axis. The analysis is a two-step approach: (1) AcceleRater generates a set of classification components based on a dataset where the acceleration data is matched to a known behaviour (so called "labeled" data), and (2) AcceleRater uses the classification components derived from the first step to analyze acceleration dataset that does not have any known behaviour linked to the acceleration data (so called "unlabeled" data). In this study, we were only interested in evaluating the *ability* of AcceleRater to classify the two different feeding behaviours in perch, and were hence only strived to derive a measure of the accuracy of the classifications. To test the accuracy of the classification processes, we first fed AcceleRater 50% of our dataset, including all information matching acceleration profile to observed behaviour (i.e. labeled data). Based on the classification components derived, we then fed AcceleRater with the remaining 50% of our dataset, but excluding information linking acceleration profile to behaviour (i.e. by removing the behaviour label we created "unlabeled" data).

Ethical Statement

All experimental protocols and animal handling in this study were approved by the Swedish Animal Ethics Board (Dnr: A-11-13).

Results

Placement study descriptive results

Sample size

A total number of 614 samples were logged during the test period, these were distributed equally between the 4 different placements. The two loggers collected data for a total of 2 hour and 48 minutes each.

Quality index parameters

Figure 10 presents how quality index parameters differed in the different placements. There was a significant difference in quality index between the data produced by a logger placed close to the heart and a logger placed in the belly, with the logger placed close to the heart having a higher probability of producing good quality data (GLMM, χ_1=84.3,p<0.01). In the logger placed close to the heart, there was no significant difference in the probability of good quality data between the logger placed with the two electrodes up towards the pelvic fins compared to the logger placed with the two electrodes down towards the dorsal fin (GLMM, p = 0.8).

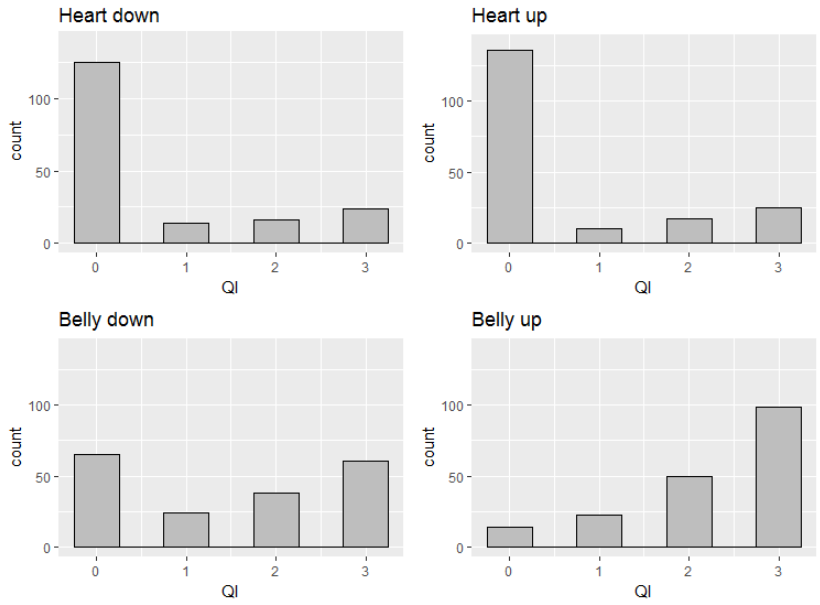

Fig. 10. Count of QI 0-3 in all four placements. QI0= Great, QI1=Good, QI2 = Fair, QI3= Poor. Heart/belly down means that the logger was turned towards the dorsal fin and in position heart/belly up it is turned towards the pelvic fins.

There was a big variation in heart rate values (BPM), including higher proportion of extreme values, for logger data with a quality index 3, whereas heart rate had smaller and approx. similar variation for data with quality index of 2 and lower (Fig. 11, see also Table 1 for mean (+- stdev) heart rates (BPM) for all combinations of QI and placement). Quality index had a significant effect on heart rate, with QI=3 having higher BPM compare to QI<3 (LMM, $F_{1.726}$=274, p<0.001, HSD Tukey p<0.01 on all comparisons between QI=3 and QI=0,1 or 2). No difference in heart rate between QI 0, 1, and 2 was found (p>0.05).

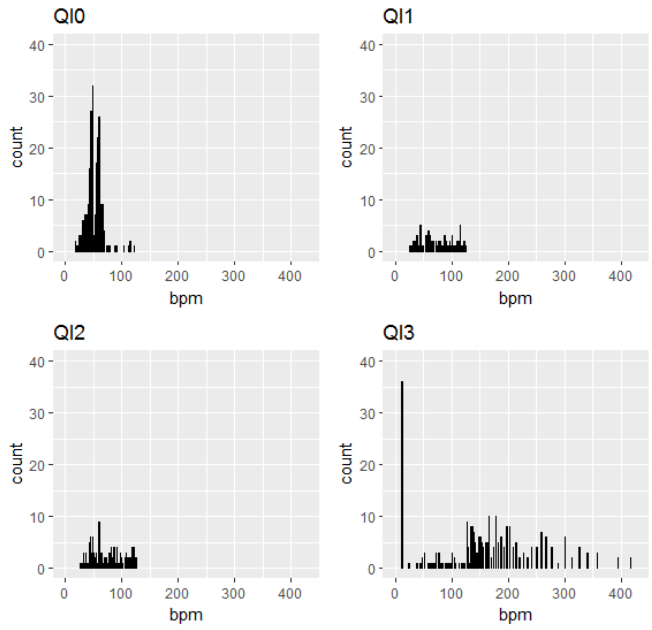

Fig. 11. Heart rate values in beats per minute (BPM) distributed within the different QI parameters.

Tab. 1. Mean BPM (StDev) in QI 0-3 in different placements.

		BellyDown	BellyUp	HeartDown	HeartUp
QI	0	51.9 (13.7)	52.0 (23.0)	46.6 (10.8)	50.0 (12.4)
	1	78.8 (28.2)	75.3 (28.6)	62.6 (31.0)	75.5 (26.6)
	2	69.4 (28.0)	81.4 (23.3)	64.8 (30.3)	67.8 (28.7)
	3	143.7 (103.3)	171.9 (81.3)	113.7 (90.6)	128.2 (97.4)

GABAergic anxiolytic drug

The total number of samples collected was 1618 samples for the control fish and 1889 for the exposed fish. There was a significant difference in heart rate between the period with handling stress compared to the period without disturbance, for the control fish (LMM, $F_{1,6}$=6.6, p<0.001, Fig. 12), with higher heart rate during the period with handling stress. For the exposed fish, there was a weak significant difference in heart rate between handling and resting periods (LMM, $F_{1,6}$=187, p=0.04, Fig. 12).

The distribution of QI differed between the data collected during the handling stress period compare to the resting period, with the proportion of QI>0 being higher during handling stress (Fig. 13).

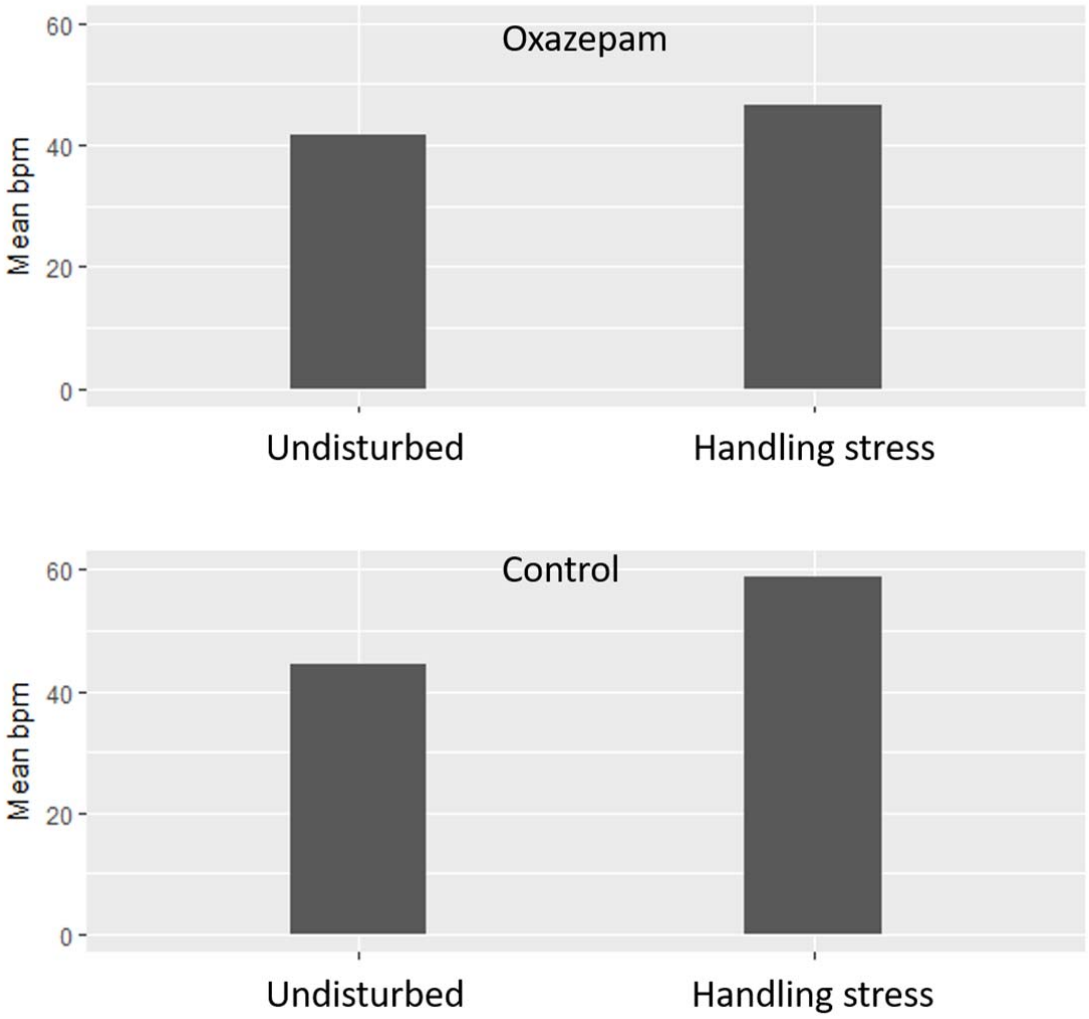

Fig. 12. Mean heart rate (BPM) when trout were handled and when they were undisturbed.

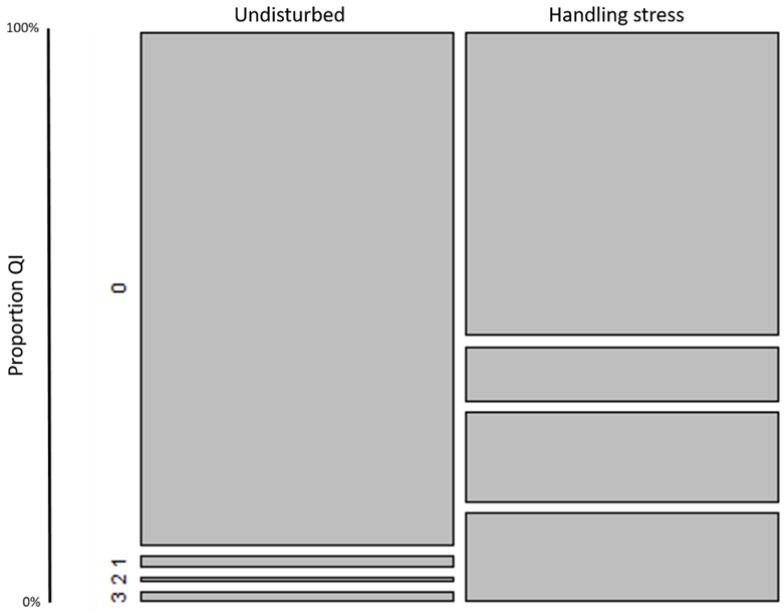

Fig. 13. The distribution of data with QI = 0,1,2 and 3 for the two different treatment periods.

DST tilt

Number of samples

The number of imitated behaviour where 62 (perch attack crucian carp) and 104 (perch forage on insect) (Fig. 14).

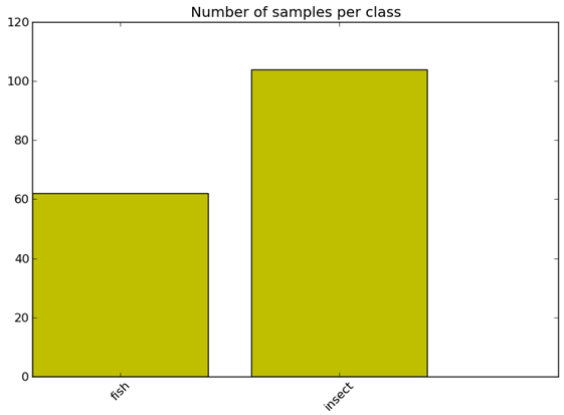

Fig. 14. Number of behaviour imitated manually. Fish refers to the behaviour when perch is attacking crucian carps and insect refers to when perch is foraging on insects.

Distinct values that can describe the attack movement performed by perch to catch another fish is three quite stable tilt values that follows of a rapid decrease of all three axes and finally a quick increase (Fig. 15).

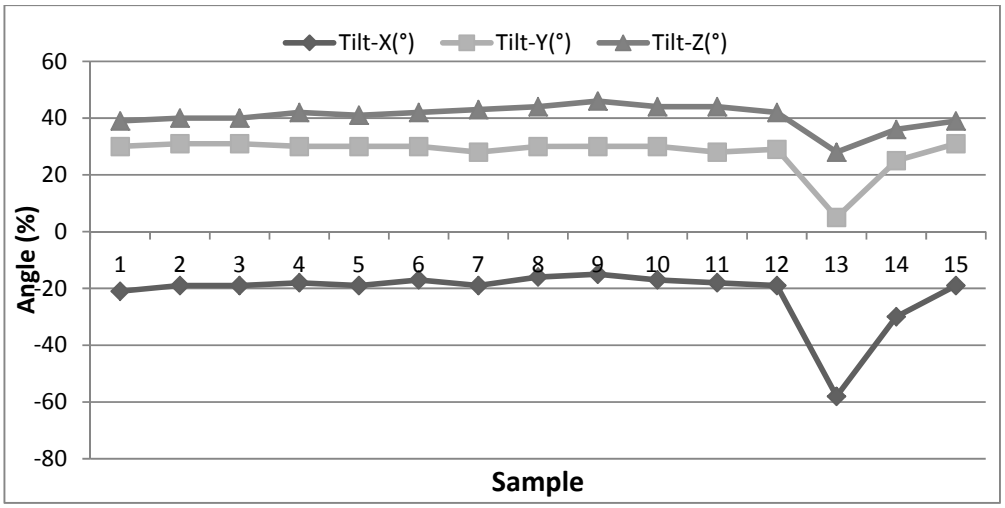

Fig. 15. The tilt values describing the attacking behaviour performed by perch on crucian carps based on 62 repetitions with a euthanized perch.

The distinct values that can be descriptive for the behaviour perch foraging on insects are a longer, less steep decrease and increase compared to the behaviour perch attacking a crucian carp (Fig. 16).

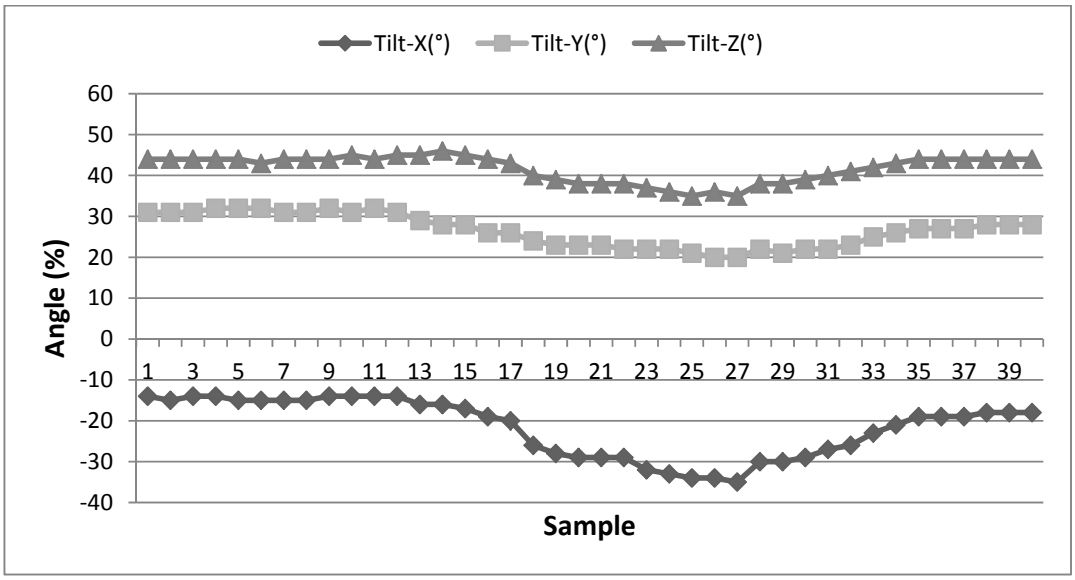

Fig. 16. The tilt values describing the behaviour of a perch foraging on an insect based on 104 repetitions with a euthanized perch.

One of the differences between the two imitated behaviours is the average of the tilt values where there is a significant difference in mean tilt X ($p = 2.42E-25$), tilt Y ($p= 1.32E-17$) and tilt Z ($p= 4.61E-07$) (Fig. 17).

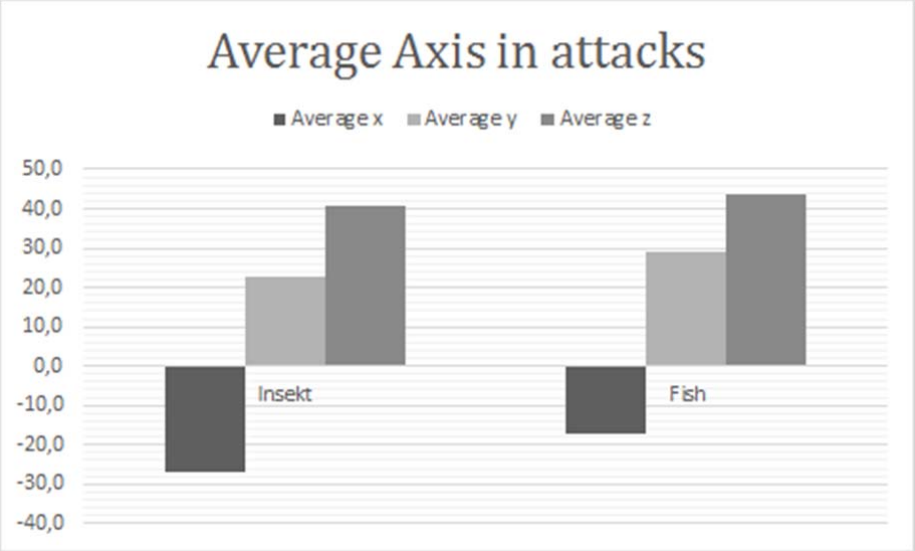

Fig. 17. Presents the average tilt angles in both foraging behaviours.

A two tailed t test shows that there also is a difference between the lengths of the imitated behaviours (p= 2.69E-42), where the imitated behaviour perch foraging on insects is longer (Fig. 18).

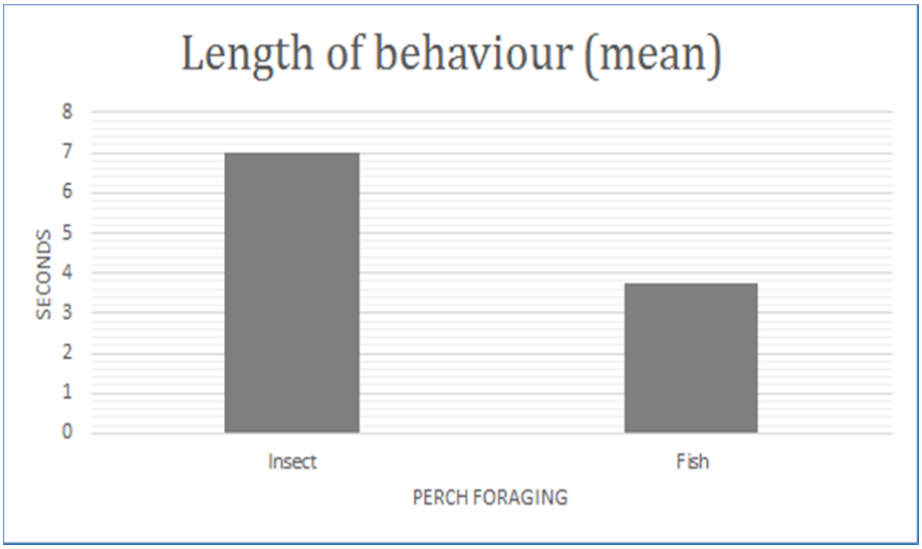

Fig. 18. The average length of the performed foraging behaviours presented in seconds.

Acceleration

The average acceleration for the behaviour perch attack on crucian carp was 9.90 m2/s and the behaviour perch foraging on insect was 9.87 m2/s. A two tailed t test revealed that there

was no significant difference in acceleration (m2/s) between the two behaviours (p= 0.18). An example of the acceleration pattern is illustrated in figure 19 and figure 20.

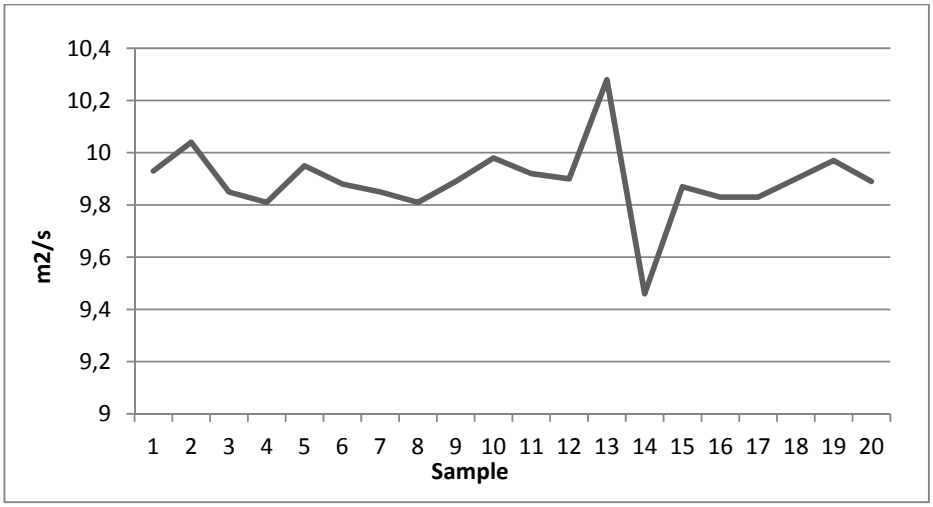

Fig. 19. The acceleration pattern describing the attacking behaviour performed by perch on a crucian carp based on 62 repetitions with a euthanized perch.

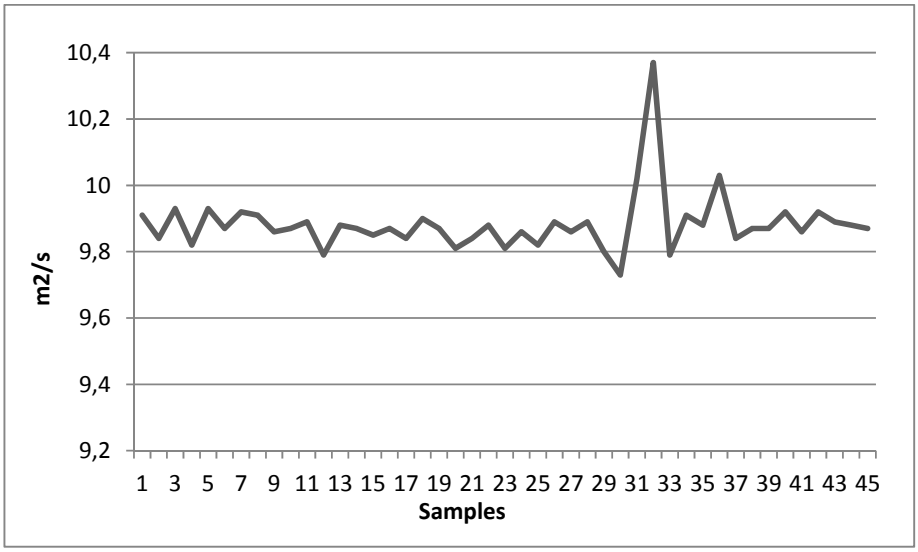

Fig. 20. The acceleration pattern describing the foraging behaviour performed by perch on insects based on 104 repetitions with a euthanized perch.

AcceleRater

All collected data are labeled. The samples used in AcceleRater are 30 labeled imitated behaviours from when perch attacked fish and 50 labeled imitated behaviours from when perch foraged on insects. AcceleRater then provided the overall accuracy of different models (Tab. 2). The program also provided information on the best fitted models to provide high recall (Tab. 3), precision (Tab. 4) and accuracy (Tab. 5) for identifying both

behaviours. After the program suggested the best model the rest of the data were used without labels (32 unlabeled behaviours when perch attacked fish and 54 unlabeled behaviours when perch foraged on insects) as a test to see how well the program provided the actual labels (Tab. 6).

Tab. 2. Overall accuracy for each model.

Model name	*% Correct*
Nearest Neighbors	97.50
Linear SVM	100.00
RBF SVM	100.00
Decision Tree	100.00
Random Forest	100.00
Naive Bayes	100.00
LDA	90.00
ANN	97.05

Tab. 3. The recall percentage for each model distributed on each behaviour.

Recall

	fish	*insect*	*Weighted average*
Nearest Neighbors	90.91	100.00	*95.59*
Linear SVM	100.00	100.00	*100.00*
RBF SVM	100.00	100.00	*100.00*
Decision Tree	100.00	100.00	*100.00*
Random Forest	100.00	100.00	*100.00*
Naive Bayes	100.00	100.00	*100.00*
LDA	72.73	96.55	*87.62*
ANN	90.91	100.00	*96.59*

Tab. 4. The precision for each model distributed on each behaviour.

Precision

	fish	*insect*	*Weighted average*
Nearest Neighbors	100.00	96.67	*97.92*
Linear SVM	100.00	100.00	*100.00*
RBF SVM	100.00	100.00	*100.00*
Decision Tree	100.00	100.00	*100.00*

Random Forest	100.00	100.00	*100.00*
Naive Bayes	100.00	100.00	*100.00*
LDA	88.89	90.32	*89.78*
ANN	100.00	96.67	*97.92*

Tab. 5. The accuracy for each model distributed on each behaviour.

Accuracy

	fish	*insect*	*Weighted average*
Nearest Neighbors	97.50	97.50	*97.50*
Linear SVM	100.00	100.00	*100.00*
RBF SVM	100.00	100.00	*100.00*
Decision Tree	100.00	100.00	*100.00*
Random Forest	100.00	100.00	*100.00*
Naive Bayes	100.00	100.00	*100.00*
LDA	90.00	90.00	*90.00*
ANN	97.50	97.50	*97.50*

Result from testing unlabeled data

The unlabeled data were tested in the different models and the models then provided own labels. The result showed that the best model for the data set would be decision tree (Tab. 6).

Tab. 6. Correspondence in percent between the labels suggested by the models and the actual labels.

	fish	insect	*Weighted average*
Nearest Neighbors	96.87	98.15	*97.51*
Linear SVM	96.87	96.30	*96.59*
RBF SVM	96.87	94.44	*95.66*
Decision Tree	100.00	98.15	*99.08*
Random Forest	100.00	96.30	*98.15*
Naive Bayes	96.87	88.89	*92.88*
LDA	93.75	94.44	*94.10*
ANN	NA	NA	*NA*

Discussion

Welfare questions answered with heart frequency loggers

This study investigated the use of a new cordless heart rate bio-logger (Star-Oddi DST micro-HRT) to measure stress in hatchery reared brown trout. The biggest advantages with

the DST micro-HRT logger compare to other heart rate loggers is that it is designed to be easy to implant in the fish as the electrodes is built into the shell of the logger (i.e. no cords needed) (Star-Oddi, 2017a). The less invasive surgery should result in faster recovery of the fish compare to the more invasive surgeries associated with other heart rate loggers. There is also less need of surgery training and skill compare to operating with traditional ECG loggers with cords (Cesarovic *et al.*, 2011).

The quality and function of heart rate loggers is heavily dependent on placement of the logger (Ponganis, 2007), and this study is the first to evaluate different locations of the logger within the fish. The present study shows that the DST micro-HRT logger should be placed as close to the heart as possible but that the direction of the electrodes does not matter. Failing to place the logger close to the heart will generate a 50% increase in poor-quality data, potentially creating biased results if not considered.

The reason for poor quality data from when the logger is placed in the belly is probably due to the long distance to the heart which makes it hard for the logger to collect a strong the ECG signal. In swimming fish the logger at the belly may also collect noise from vibrations in the water or from undulation movements of the fish, which likely are more pronounced in the belly (mid part of the fish) compare to closer to the heart (head part of the fish).

Although this study was made on trout, I believe the results would be applicable to most fish species with similar physiology and morphology as trout. Hence, based on these results, I recommend that care should be taken when placing these tags in fish, and that future user of the loggers should consider the following surgical protocol: The logger should be attached close to the heart, and fixed with sutures so that the logger can't move when the fish swim. This can be done by using the small hole positioned in the front of the logger. If you insert the side without the holes towards the heart, the side with the holes should be positioned straight under the incision. It would then be possible to attach the logger with suture in the flesh wall on either side of the incision.

Despite several attempts to get clarification from Star-Oddi on how the quality index parameter is derived, it's still unknown. However, the larger variation in heart rate, as well a larger proportion of extreme values (BPM>100) for measurements with QI = 3 compare to measurements with QI 2 and lower, truly indicate that QI = 3 data stands out as more unreliable. This fact also validate our classification of data into poor (i.e. QI=3) and good (QI<3). QI=3 consistently generated higher (often unrealistic high) PBM values compare to QI>3, suggesting that poor quality data tend to overestimate the HRT. Although not significant, QI 1 and 2 still produced higher heart rate values compared to QI = 0, something that should be considered if setting a cut-off at QI = 2 (i.e. discard all data with QI<3) which is currently recommended by the manufacturer.

This study also showed that the Star-Oddi DST micro-HRT logger is capable of detecting changes in heart rate due to handling stress and drug exposure. The result showed that handling stress did not induce increased heart rate for fish exposed to oxazepam, but did increase the heart rate on the control fish. Mean heart rate during the undisturbed phase was close to 40 BPM for both the exposed and the control fish, which is similar to reported values from other studies on heart rate (Aho & Vornanen, 2001). Interestingly, when the control fish was undisturbed the heart rate still fluctuated more than the exposed fish. This

may be due to the exposed fish was being less active than the control fish, and hence moved around less which may create less noise and disturbances to the logger. When handled the heart rate was considerably higher for the control fish, indicating higher stress levels. However, the heart rate varied greatly (~20 BPM to ~120 BPM) for both treatments during the handling stress, and this in combination with a higher proportion of low quality data during handling, indicates that although the logger likely correctly are able to measure the mean heart rate during handling, the data may also be less reliable. Some of these values may be inaccurate and this may again suggest that also values with QI= 1 or 2 should be excluded from the data before analysis.

Heart rate is a direct physiological parameter indicating stress level (Clark *et al.*, 2010; Cooke *et al.*, 2002), and the fact that these simple to apply cordless heart rate loggers are able to distinctly capture change in heart rate following handling stress and drug exposure bode well for future studies on fish welfare, especially in a hatchery context.

It is also notable that fish exposed to oxazepam had a much less acute heart rate response to handling stress compare to the control fish, and my study is the first to show this response using an easy to apply heart-rate bio-logger. Pharmaceuticals have been present in natural freshwater systems for at least 50 years, and how this affects fish populations is still not properly investigated (Brodin *et al.*, 2014). It has been shown that pharmaceuticals affect behaviours that are important for the ecosystem functioning and fish fitness (Brodin *et al.*, 2014), but the underlying physiological mechanism and causal chains generating these effects are still unknown.

Based on my results, I propose that the DST micro-HRT loggers can be used in ecotoxicological studies to examine how different species are affected by pharmaceuticals. If oxazepam is reducing stress response, how does this affect the predator prey interaction? Can oxazepam be used in fish farms to improve welfare? One example of a study design could be to tag farmed fish with DST micro-HRT loggers, then contaminate the fish with oxazepam. All routines towards the contaminated fish could be as generally carried out at the fish farm. The result could present factors like heart rate, growth, meat quality and heart rate changes within different events. These factors could be compared to a control group without the contamination. The gained information from the result could help to answer if oxazepam could be used to increase welfare in farmed fish or to answer what handling event seems to be the most stressful for the fish.

Locomotory activity has been shown to increase heart rate in fish (Cooke *et al.*, 2002), and although locomotory activity was not a factor in this study (both fish were handled in the same way), it may be a confounding factor in other studies where you cannot control for or measure swimming activity. However, if you use the logger together with e.g. an accelerometer, it may be possible to decide if changes in BPM depend on altered physiological stress response or changed locomotory movement.

It should be noted that the sample size for the placement study was small, i.e. only two fish were tested. The results from this study should hence be viewed in the light of this. If this study should be repeated, sample size should be increased at least 5 fold. Also the sampling time should be longer so that the handling events could be less frequent. Then also the time of recovery could be an important parameter in the result. In this study the handling was to frequent so that the BPM never had time to go down before going up again.

In the future the DST micro-HRT logger could be used to for example forecasting stressors in fish farms or to forecasting the consequences of global warming. This is possible because the global warming is increasing the cardiorespiratory activity in ectothermic animals like fish (Ekström *et al.*, 2016; Sandblom *et al.*, 2016). Studies have been made by using different HRT loggers to compare heart frequencies in different water temperatures and on different fish (Ekström *et al.*, 2016; Sandblom *et al.*, 2016). There have also been studies showing that the heart rate increases due to acute stress are higher in fish in warmer water temperature than in colder (Ekström *et al.*, 2016). This knowledge can be used as a factor to take the temperature into account when comparing heart rates.

Basic ecology and acceleration Bio-loggers

This study show that the use of DST tilt logger can be used to distinguish between different feeding behaviours in Eurasian perch, by using automatic ordination classification analysis provide through the software AcceleRater. This finding has several implications in both ecology and aquaculture. Being able to get detailed information on the foraging behaviour of wild fish is rare, and may give interesting insight into life-history events such as ontogenetic shifts. For Eurasian perch for example, switching from being an insectivore to becoming piscivore is a landmark change in life, radically impacting growth and survival (Gerking, 1994).

Such change may even create a trophic cascade in an entire aquatic ecosystem, and knowing when and under what circumstances individual fish makes the transition to piscivory would provide much needed knowledge about ecosystem function (Persson *et al.*, 2003). Further, collecting data on what, when and how much a fish eat in the wild would have the potential to greatly improve bioenergetics models in fish, and hence better understand energy transfer and growth of the whole ecosystem (Hartman & Hayward, 2007). Such approach would be more solid if one also could determine which feeding attempts where successful and which were not. This would likely be detectable using acceleration loggers, as a successful feeding event would involve some handling time of the prey, likely separating the acceleration profile compare to an unsuccessful event. Given the high rate of success to distinguish between feeding behaviours in this study, I do believe there is a good potential in exploring this further (Van Deurs *et al.*, 2017). Accurate foraging information from the wild could potentially be combined with spatial data of the fish, using fine-scale positioning techniques such as acoustic telemetry. This would make it possible to not only knowing when and what the fish eat, but also *where* the fish eats, something that could give valuable insights into what drive habitat choice in fish. In aquaculture acceleration bio-loggers can be used to measure the energy expenditure and hence making it possible to for example calculate optimum feeding amounts.

There are different ways of explaining the difference between the two behaviours in this study. The data was used to present how the tilt values played out differently for both behaviours (Fig. 19, Fig. 20), how the mean value in the 3 axis differed between behaviours (Fig. 21) and how the length of the two behaviours differed (Fig. 22). The DST tilt also provides with acceleration. The acceleration is in this study presented in the mean acceleration and also in how the acceleration pattern distributes throughout the behaviours (Fig. 23, Fig 24). The result presented a difference between the two behaviours in mean tilt and mean time but not in acceleration.

One major disadvantage with bio-logger in general when studying fish in the wild is the need to recapture the fish to collect the data. A new, transmitting acceleration logger has

recently hit the market, promising to remotely collect data from free-swimming fish (ThelmaBiotel, 2017). The logger is calibrated to recognize certain behaviour profiles reflecting certain behaviours, and once the logger detect such profile, it transmits a simple "detected" signal to the receiver. This way of circumvent the problem with narrow bandwidth under water is innovative and have potential to further expand the usability of acceleration loggers (de Almeida *et al.*, 2013).

The results from this study showed that no model gave 100% correct answer (Decision tree = 99%). This may be due to that the two feeding behaviours sometimes were similar. Those behaviours that got assigned wrong labels by AcceleRater were almost the same in each of the different models tested by AcceleRater, indicating that these particular observations stood out as different. The program provides information on the best fitted models considering high recall, precision and accuracy for identifying both behaviours. Accuracy shows the probability of that a sample in the labeled data is being assigned correctly to the specific behaviour. Precision answers if the probability that an assigned behaviour in the labeled data is indeed this particular behaviour and recall is presenting the probability that a sample with a particular behaviour in the labeled data will be correctly classified as this behaviour. All models provided high % for both behaviours and 5 models provided 100% on recall, precision and accuracy on both behaviours.

It is important to consider the shape and size of the logger when designing a study. The weight of the logger should not be more than 2-5% of the weight of the animal´s total bodyweight (Fehlmann & King, 2016). In this study, logger size and weight was less important due to having a dead perch as the study object. Modern acceleration loggers are rapidly getting smaller, but often at the expanse of battery life and memory capacity. This is an issue if one wish to study rapid movements like attack or escape movement's which demands sampling at higher frequency. The acceleration logger used in this study is considered relatively large, but still it took only about 3 hours before the memory was full if set to sample at the highest frequency. As fish tagged with an acceleration logger will need at least a few days to recover and start to eat again, making it important to program the logger to delay the start of the sampling. Improvement of memory capacity would create a much broader use of acceleration loggers, and is likely the most critical area for technical improvement.

Until the loggers get much smaller with better memory and battery life, the use of DST tilt logger should be restricted to larger fish species and set to sample with lower sampling frequency. In the DST tilt logger sampling of tilt and acceleration could also be combined with sampling of depth and temperature. Then for example you could study how temperature and depth affects feeding behaviour. This could help answer important questions about the climate change and how it effects fish populations.

Conclusions

Bio-loggers have a big potential for providing scientist with information to increase the knowledge about fish behaviour and physiology both in captivity and in the wild. In this study the DST micro-HRT logger purchased from Star-Oddi was tested by examining where to place the logger inside the fish to get the most accurate data. The result showed that the placement is important because the logger receives the heart frequency at a bigger extent when placed close to the heart than when it is placed in the belly. Another investigation was made by performing a small pharmaceutical study to find out how the logger functioned and if it could be used to examine the effect of a GABAergic anxiolytic

drug on the fish heart frequency. The pharmaceutical part showed that the increase in heart rate was smaller in the individual contaminated by the drug compared to the individual that was not contaminated. In addition to these two investigations a DST tilt logger purchased from Star-Oddi was examined. The logger was able to record behavioural data that could be used in a program to create supervised learning of behavioural modes with 99% accuracy. The issues in the study were linked to the restriction of the loggers. In the DST micro-HRT logger the limitation was primarily the battery life and in the DST tilt logger the limitation was primarily the memory capacity. For future studies with the DST micro-HRT logger the placement should be prioritized. If it is going to be used under a longer period it should be set to sample less frequent to put less stress on the battery. Future development should be an increased battery capacity. Future development of the DST tilt should be an increased memory capacity.

Acknowledgements

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Appendix

Failed studies

- Pike were marked with acoustic telemetry tags and acceleration loggers and released in a pond in Åmsele situated in northern Sweden. All other fish in the pond were also marked with positioning tags and were indirectly meant to be a part of this investigation. The aim was to study position and acceleration in combination to later predict behavioural event like attack or escape. A second part of this study was to study behaviours of pike in an indoor tank to get the acceleration profile of attacking movements. Two pikes were tagged with DST tilt loggers and all feeding occasion was filmed. The collected data from the indoor tank was later going to be used to get the data from the pike in Åmsele labeled. This could be done by inserting the labeled data from the pike attacks in the indoor tanks into AcceleRator. Then insert the unlabeled dataset collected from the pike in Åmsele. The new labeled data would then also have a timestamp and could be linked to when, were and in contact to what other individual the attack behaviour was performed. The issues were that the pike in the indoor tank refused to eat. This may be because of stress to the new environment. The pike that was tagged and released into the pond in Åmsele was never cached so the data could not be collected.
- Two perch and one roach were tagged with DST micro-HRT loggers with purpose to study how the heart frequency changed when the fish were exposed to stressors. This study was performed by first letting the fish recover from surgery for a week. Then when the logger started to sample the perch and roach were stressed 3 times over a timeframe of an hour, by knocking on the aquarium glass for 15 times. The rest of the time the fish were undisturbed. The issue of this study was that the loggers were incorrectly inserted so that the data had a lot of QI 3 values and hence of poor quality.
- Perch were tagged with DST tilt loggers. They were after post-surgery recovery filmed while being handled with a ring net or being exposed to a predator (pike). The aim was to explore if the DST tilt logger could detect changes in behaviour performed by perch when stressed. The issue was that the bio-logger likely was too large for the perch, and the perch did hence get affected by the logger and hence did not behave natural. The study was hence terminated early to reduce stress on the fish.
- Filmed behaviour of the perch feeding on crucian carps and insects were supposed to be used by a company to create specially designed loggers that could be programed to recognize certain pre-set behaviours. The issue was that the company thought that the behaviour were to similar and denied the proposal.

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Författare: Ruben Bloemsma

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