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Thinking outside of the box:

Monitoring heart rate and body temperature in rainbow trout, *Oncorhynchus mykiss, using a bio-logging system*

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Ämneslärarprogrammet

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Abstract

Conducting true ecophysiological studies on fish has been difficult due to there being few methods for monitoring vital responses in natural environments. Techniques such as bio-telemetry and bio-logging show great promises in ecophysiological research, bringing researchers and animals out of the lab and one step closer to monitoring a fish's physiology in its natural habitat. One potential with this approach is the possibility to conduct research on several fish at once during a long time period something that is not possible using the traditional hard wired monitoring techniques. This method also opens up possibilities for using larger tanks and even mesocosm settings when studying fish. In this study we test the capability for STAR-ODDI's DST milli-HRT bio-logging system to monitor heart rate and body temperature in rainbow trout, *Oncorhynchus mykiss*. With this system, we were interested in investigating laboratory induced stressors while using heart rate as an indicator for distress. Our results show that grouping fish together is a surprisingly powerful stressor as it elicits a significantly stronger heart rate response than netting and chasing the fish. We also found that the fish were quite susceptible to confinement stress, where fish housed in a small tank traditionally used for hard wired studies (with very limited space for the fish to swim around in), had a significantly higher heart rate compared to fish housed in a larger tank. We also found that using a traditional chasing protocol to stress the fish, the scope of the heart rate (maximum – minimum heart rate) is underestimated by nearly 30% compared to using forced social interaction. These results give new insights in the stressful effects of laboratory environments on research fish and shows that the new STAR-ODDI system can be used successfully for these types of studies.

Index

Abstract 2
Introduction 4
Materials and methods 5
Study Outline Summary 5
Animals Used 6
Ethical Statement 6
Programming DST milli-HRT 6
Preoperative Procedure 6
Surgical Procedure 7
Experimental Procedure 8
Data analysis and statistics 9
Results 9
Discussion 16
Stressor Response 16
Conclusion and application in future research 17
Application in biology didactics 18
Acknowledgements 19
References 19

Introduction

Most ecophysiological studies aim to measure vital responses of animals in their natural environments with as little disturbance as possible. Within fish biology it has been difficult to perform true ecophysiological studies since most methods for measuring physiological responses have been quite invasive and demand that the fish is physically connected to the equipment which severely limits the behaviors of the fish. However, novel biotelemetry and bio-logging equipment have lately made ecophysiological studies possible also for fish (Armstrong et al., 1989; Lefrançois et al., 1998; Clark et al., 2010; Gräns et al., 2009b; 2010).

Biotelemetry is a remote measurement of an animal's physiology measured and transmitted to a receiver where the data can be observed and interpreted (Cooke et al., 2004). Many early commercially available telemetric systems were not fully implantable in the body of the animal and thus parts of these systems were therefore externally attached to their bodies, leaving open wounds (see Axelsson et al., 2007). Modern biotelemetric devices are small enough to be surgically inserted into the animal where the wound can be closed to minimize the risk of infections (Axelsson et al., 2007; Gräns et al., 2009b). With the bio-telemetric technology available today it is possible to measure temperature, swim speed, heart rate, blood pressure, blood flow (e.g. cardiac output, gastrointestinal blood flow) in free swimming fish (Lefrançois et al., 1998; Rand & Hinch, 1998; Hinch et al., 2002; Healey et al., 2003; Gräns et al., 2009b; Gräns et al., 2010). Studies show that the usage of bio-telemetric techniques can lower confinement stress (see Gräns et al., 2009b). When using biotelemetry it is possible to study physiology in more natural conditions since the technique is wireless (Armstrong et al., 1989; Briggs & Post, 1997; Axelsson et al., 2007). However, all telemetric devices rely on being able to transmit a signal to some sort of receiver. This means that the animal still has to stay in range of the receiving device. Long range biotelemetry, which does exist, relies on satellite in order to transmit data. If a fish (which is being studied with the use of satellite biotelemetry) disappears into the water, the signal is lost. One solution to this problem has been to use pop-up tags which automatically come up to the surface when the animals dies, or after a fixed period of time, and then starts transmitting the data via satellite (Graves, et al., 2002).

Bio-logging is a method which involves storing data on a recording device for later retrieval (Cooke et al., 2004). These recording devices can be engineered to monitor the same processes as bio-telemetric devices without having to rely on the transmission of data. One downside to these bio-logging systems is that they can be difficult to retrieve since they have to be manually removed from the fish, alternatively float up when the fish dies. Another drawback is that they often give limited possibilities to optimize the signals during implantation as the signal cannot be observed "online".

The DST (data storage tag) milli-HRT from STAR-ODDI, shown in **Fig. 1**, is a small implantable bio-logging device with built-in electrodes which can measure and record heart rate as well as the body temperature of different animals. The DST milli-HRT implant is 40 mm in length and weighs 11.8 g, with a battery life of approximately 10 months. The battery time strongly depend on the logging frequency and the water temperature. The advantage of this is that the range is limitless, since the data is stored onto the device. The device has been constructed mostly for studying smaller mammals and has never previously been tested on fish.

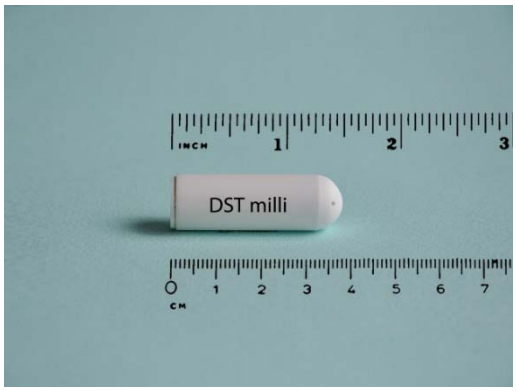

Fig. 1 The DST milli-HRT heart rate and temperature logger from STAR-ODDI (Star-Oddi).

Heart rate has been used successfully as a stress indicator in several studies (see Cooke et al., 2002). Variations in heart rate are also known to indicate distress in animals (see Gräns et al., 2014), including some types of fish (Campbell et al., 2004). Heart rate is a central factor in the cardiorespiratory system of all vertebrates and is sensitive to almost everything an animal does voluntarily or is exposed to in its environment. Several previous studies have therefore used heart rate as an indicator for other variables such as: activity, food intake, digestion and predator presence (Altimiras & Larsen, 2000; Gräns et al., 2010; Höjesjö et al., 1999). These correlations should however be made with caution as other studies have shown that heart rate modifications are complex and can easily be misinterpreted (Priede & Tytler, 1977; Thorarensen et al., 1996; Lefrançois et al., 1998).

The aim of this study was to monitor heart rate for assessing the effects of different stressors on rainbow trout (*Oncorhynchus mykiss* L.) in a laboratory environment. We hypothesized that surgery, confinement, netting, chasing and social interactions would all be perceived as stressful by the fish and thus give higher heart rate compared to when the fish are isolated and undisturbed. In this study, the potential usage of the DST milli-HRT in ecophysiological research on fish was also assessed, more specifically its ability to monitor heart rate during various situations.

Materials and methods

Study Outline Summary

This study was divided into three experiments which were designed to test different stress factors on the fish while being held in captivity, using heart rate as an indicator for stress. The purpose of the first experiment was to investigate the effects on heart rate when isolating fish into separate tanks, netting the fish, placing all the fish in the same tank and chasing the fish. The second study was composed of a confinement experiment, where all the fish were held in separate sections of a common tank. The purpose of this experiment was to analyze the stress effect of a black box (a strictly confined area commonly used in studies of fish physiology) on the animal.

The recorded signal strength during experiment two was found to vary immensely which made several measurements unusable. Therefore, a third experiment was conducted in order to evaluate which implant positioning would give the clearest and strongest signal. The details of each experiment are outlined below.

Animals Used

A total of 20 rainbow trout, *Oncorhynchus mykiss*, ranging from 525 to 990 g (mean±S.E.M: 630±98) in size where used in this three-part study. The fish were purchased from Antens Laxodling AB, Alingsås, Sweden. Before the experiments, all fish were held in two 2 m^3 tanks constantly supplied with aerated fresh water (10°C) where they were fed twice a week with commercial dry trout pellets.

Ethical Statement

All experimental protocols were approved by the Ethical Committee of Gothenburg (permit 177-2013).

Programming DST milli-HRT

Experiment 1

A total of seven implants were used in this experiment. The implants were all programmed to measure temperature and heart rate in three different intervals. The first interval was set to measure every 10 minutes for 90 hours including three saved buffer sequences for evaluating the quality of the signal. The buffer sequence consists of a ten minute recording interval where the entire EKG signal is saved. During the second interval, which measured during the day of the netting and grouping of the fish, measurements were made every minute for 24 hours including two saved buffer sequences. The final interval was set to measure heart rate every 10 minutes for 55 hours including one saved buffer sequence. The total measurement time was seven days.

Experiment 2

A total of 11 implants were used in this experiment. The implants were programed to measure temperature and heart rate every ten minutes for 144 hours including 12 saved buffer sequences. As we only had seven implants, this experiment was divided into two parts with four fish used during six days and another seven fish used the following week for another six days.

Experiment 3

One DRT milli-HRT implant was used in this experiment. The implant was programed to measure heart rate every two minutes including a two minute saved buffer sequence measured every eighth minute. For this experiment the implant was slightly modified by gluing a small silicon tube, approximately 1 mm in length to the flat end of the implant so that this end could be anchored to the fish.

Preoperative Procedure

The fish were anaesthetized individually by placing them in a small container with water containing 150 mgl^{-1} MS-222 (ethyl 3-aminobenzoate methanesulphonic acid, $C_{10}H_{15}NO_5S$) buffered with 300 mgl^{-1} $NaHCO_3$. When the fish stopped ventilating it was placed on a bed of water-soaked rubber foam on a surgical table. During surgery the gills were continuously flushed with aerated 10°C fresh water containing 75 mgl^{-1} MS-222 buffered with 150 mgl^{-1} $NaHCO_3$ to ensure that the fish was kept anaesthetized during the surgery.

Surgical Procedure

Insertion of DST milli-HRT

Experiment 1 & 2

A midventral 30 mm incision was made approximately 20 mm posterior to the pectoral fins (see **Fig. 2**). The DRT milli-HRT implant was inserted posterior to the pericardium, in-between the liver and the muscle tissue. The implant was anchored to the muscle and the incision was closed with sterile 3/0 silk sutures. The fish was placed in a small freshwater tank for recovery where ventilation and consciousness could be monitored and ensured before the fish was transported to the experiment set-ups.

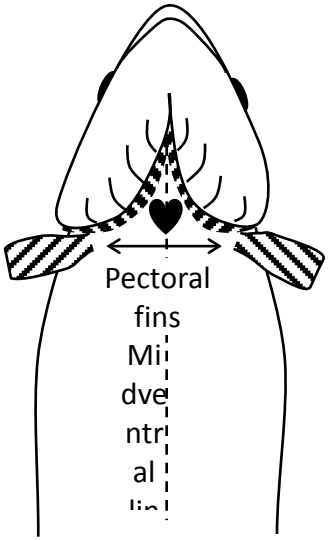

Fig. 2 The ventral side of a rainbow trout, showing the position of the heart, the pectoral fins and the midventral line.

Experiment 3

A midventral 30 mm incision was made approximately 40 mm posterior to the pectoral fins. The DRT milli-HRT implant was inserted and positioned in the proximity of the pericardium, in-between the liver and the muscle tissue, away from the point of incision. The implant was rotated in a different way each time (see **Fig. 3**). The implant was anchored to the muscle at the anterior end of the incision before the incision was closed with sterile 3/0 silk sutures. The silk suture was cut with surgical scissors, the implant was removed and then the insertion process was repeated with the implant in a different rotation. When a "best position" had been concluded one fish was placed in a small freshwater tank where the recovery could be monitored (see the surgical procedure for experiment 1 & 2 above) before the fish was transported to the tank used in experiment 1. This last part was done in order to compare the signals received from the surgery table (where the fish is anesthetized) with the signals received from the tank (where the fish is awake and moving).

After the completion of the experiments, all fish were killed with a sharp blow to the head in order to retrieve the bio-loggers.

Experimental Procedure

Experiment 1

Seven rainbow trout ranging from 540 to 990 g (mean±S.E.M: 640±158 g) were used for this experiment. The DST milli-HRT was surgically implanted using the surgical procedure outlined above. The same seven fish were used in all three parts of the first experiment.

Experiment 1.a. – recovery from surgery and isolation

Within a time span of five minutes directly proceeding surgery, each fish was transported to and placed in a separate circular 500 liter tank (tanks used for the commercial farming of salmonids) supplied with aerated fresh water (10°C). The fish were left undisturbed and without food for 92 hours.

Experiment 1.b. – Netting

Each individual fish was netted and held in air for 60 seconds before it was placed back into the same tank from which it was taken from. The fish were then left undisturbed in their tanks another 7 hours.

Experiment 1.c. – The effects of grouping on heart rate

All seven fish were netted and placed together in one of the tanks. The resident fish of that tank was also lifted out with a net and then placed back into the same tank to give the same effect. The fish were left undisturbed together in the tank for 69 hours.

Experiment 1.d. – chasing

The chasing was conducted while all seven fish were grouped in the same tank. Two nets were placed into the tank and the fish were encouraged to exercise for 5 min.

Experiment 2 – confinement/black box

A total of eleven rainbow trout ranging from 525 to 726 g (mean±S.E.M: 634±54) were instrumented with the DST milli-HRT bio-loggers. The fish were placed in an experimental tank divided into seven separate 44 cm × 10 cm × 15 cm (L×W×H) slots, where each fish had a slot for itself. Each slot was covered with a thin lid and weights to ensure darkness and eliminate the possibility for the fish to jump out. The tank was continuously supplied with aerated fresh water (10°C). Since there were eleven fish total and only seven slots in the tank, the experiment was carried out in two rounds. The fish were left in the tank for 144 hours (six days) in both the rounds.

Experiment 3 – implant positioning

Two rainbow trout, 555 and 582 g in size, were used for this experiment where each fish was used as its own control. The DST milli-HRT bio-logger was implanted into a fish in five different positions (see **Fig. 3**) at different times, where the implant was left for at least 10 minutes every time. This experiment was done in order to establish a controlled implant placement with the aim of aiding future studies in optimizing the quality of the signal.

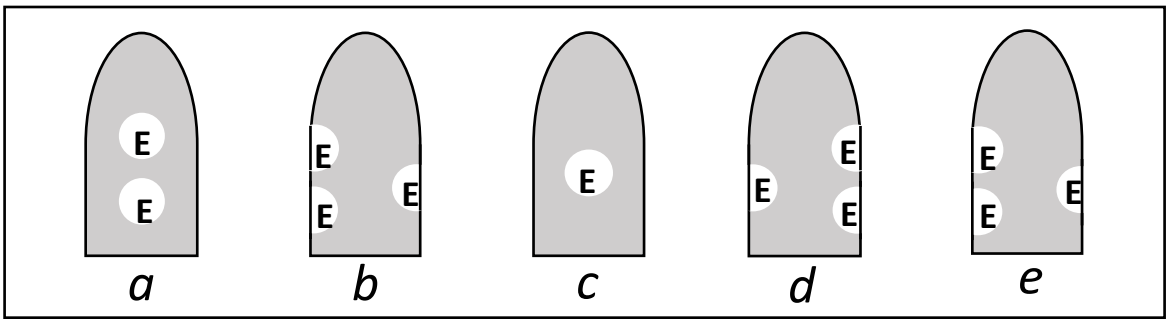

Fig. 3 The DST milli-HRT implants where positioned in five different variations (shown from the ventral side of the fish) when testing the signal strength. *e* differs from *b* since it was placed 20 mm posterior from that of the positioning of *b*. The electrodes on the implant are marked with an *E*.

Data analysis and statistics

In the experiments, the following variables were analyzed: maximum heart rate after a stressor, minimum heart rate during rest, scope for heart rate, daily and hour by hour average. Data retrieval was done using the application software for Star-Oddi logging system (Mercury v 3.21, Reykjavik, Iceland) and further data analyses was done in Microsoft excel 2010. Importantly, only readings that had not been marked with any form of quality errors by the Mercury software was used for the analyses. Maximum heart rate after a stressor was set as the single highest value 2 h following the different stressors. Minimum heart rate for a fish was the single lowest recording during the whole experimental period. Scope for heart rate was calculated as the difference between the maximum heart rate (after both grouping and chasing) and the minimum heart rate. The daily averages included all recordings of each fish for every recording day except for day 4. On day 4, hour by hour averages were calculated using the mean heart rate of all recordings for each fish. SPSS 20 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis.

A general linear model was used for comparison of the dependent variable (heart rate) among and between treatments. The different individuals where set as subjects with an unstructured repeated covariance structure. The repeated variables were: the different stressors (netting, grouping and chasing) for the maximum heart rate analyses, the time of day for the hour by hour analysis and the different days for the daily average analyses. In the day by day analysis, the housing condition (single in a tank or in a group of seven) was also included as an explanatory factor. The explanatory factors and their interactions were compared using a Sidak confidence-interval adjustment. A two tailed paired t-test was used for comparison between scope for heart rate using grouping or chasing as the mean to produce the maximum heart rate. A two tailed t-test was also used for comparison between fish housed alone in the black box tank and fish house alone in a large tank. All data was tested at a significance level of $p<0.05$.

Results

Experiment 1

Maximum heart rate after different stressors

Heart rate was significantly different between all three stressors (see **Fig. 4)**. The mean minimum heart rate was 22±1 bpm and was obtained using the lowest recorded value for each fish. The highest mean

heart rate at 83±4 bpm was measured after the grouping of the fish while the lowest maximum heart rate at 65±3 bpm was measured following the chasing protocol.

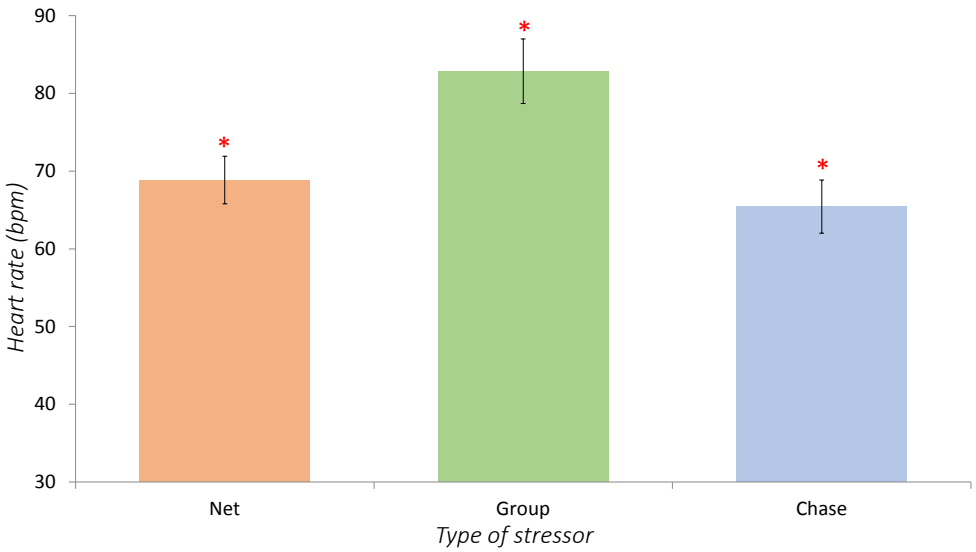

Fig. 4 Maximum heart rate (beats per minute) during three different stressors. The asterisk (*) shows that all *heart rate* differed significantly from one another, $p<0.05$. n=7 for all three stressor types.

Increase in heart rate

The increase in heart rate was calculated by subtracting the mean minimum heart rate (22±1 bpm) from the maximum heart rate after either the fish were grouped or after being chased (see **Fig. 5**). Grouping fish produced a significantly higher (28%) heart rate increase compared to when the fish where chased for 5 min.

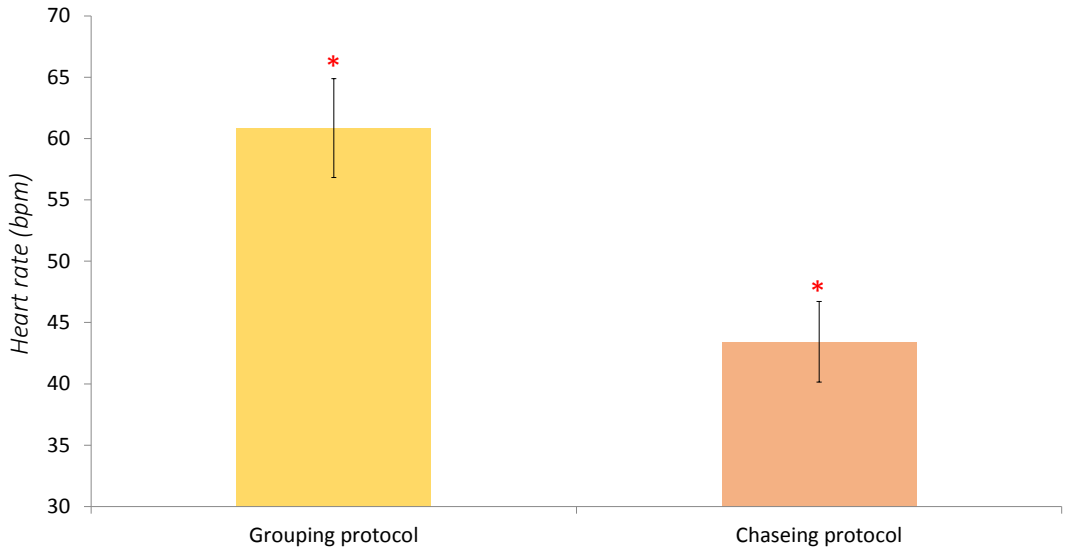

Fig. 5 The increase in heart rate (maximum heart rate – minimum heart rate) when using two different stress-protocols (grouping and chasing). The asterisk shows that the effect of the two stressors differ significantly from one another, $p<0.05$. n=7 for both protocols.

Mean heart rate day by day

The mean heart rates per day during the experiment have been mapped out in **Fig. 6**, with day 4 being excluded since this was the day that the netting and the movement to a common tank took place. Heart rate on day 5 was significantly higher than all other days (**Fig. 6**). The greatest difference existed between day 5 (54±2 bpm) and day 1 (43±2 bpm) where heart rate increased by ca. 12 bpm. No other days had significantly different mean heart rate values when compared at the $p<0.05$ level. Though when also including housing conditions a) one fish per tank (day 1-3) and b) in a group of seven (day 5-7) as factors in the statistical model, fish had significantly higher heart rate when housed in a group compared to when housed alone (see **Fig. 6**).

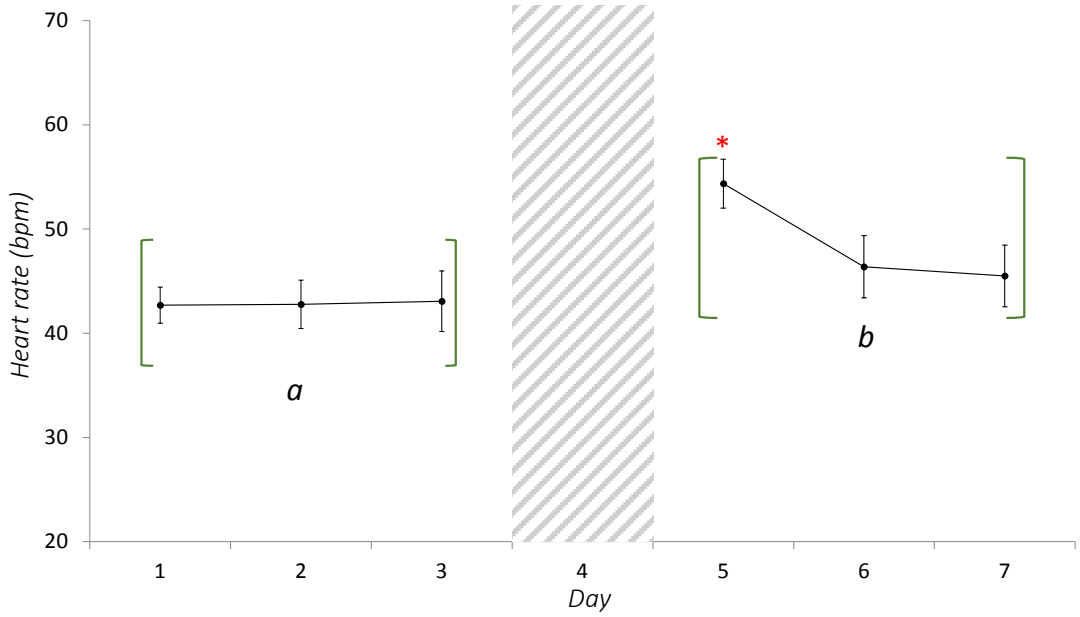

Fig. 6 Mean heart rate per day for the duration of the experiment. Day 4, indicated by the shaded segment, is excluded from data and analyzed separate as both the netting and the grouping of the fish were done on this day. The asterisk marks that the heart rate is significantly different at day 5 compared to all other days of the experiment, $p<0.05$. *a* indicates that the fish were kept isolated from one another and *b* indicates that the fish where grouped in the same tank. n=7.

Mean heart rate on day 4 *hour by hour*

Assessing mean heart rate hour by hour during day 4 shows in **Fig. 7** how heart rate increased after both netting (a) and grouping (b) of the fish. **Fig. 7** also shows that there was a significant difference between mean heart rate two hours following netting and at least seven hours following grouping in comparison with the first hour.

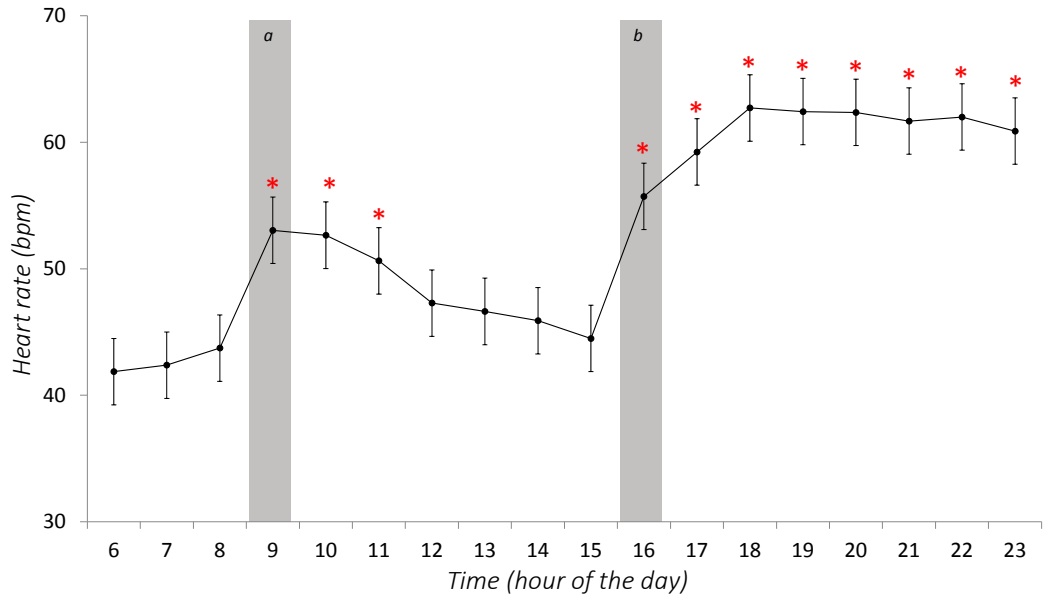

Fig. 7 Mean *heart rate* every hour during an 18 hour period. Shaded area *a* represents the point where the fish were netted, and shaded area *b* represents the point where the fish were grouped in the same tank. The asterisks (*) marks hours that are significantly different from the initial hour, $p<0.05$. n=7.

Experiment 2

In **Fig. 8** a comparison is made between the mean heart rate of individuals housed in a confined experimental black box and the individuals who swam freely in the large tanks from experiment one. Fish housed in a large tank had a significantly lower heart rate (43±3 bpm) compared to the fish housed in the black box (53±2 bpm) which is a difference of ca. 10 bpm.

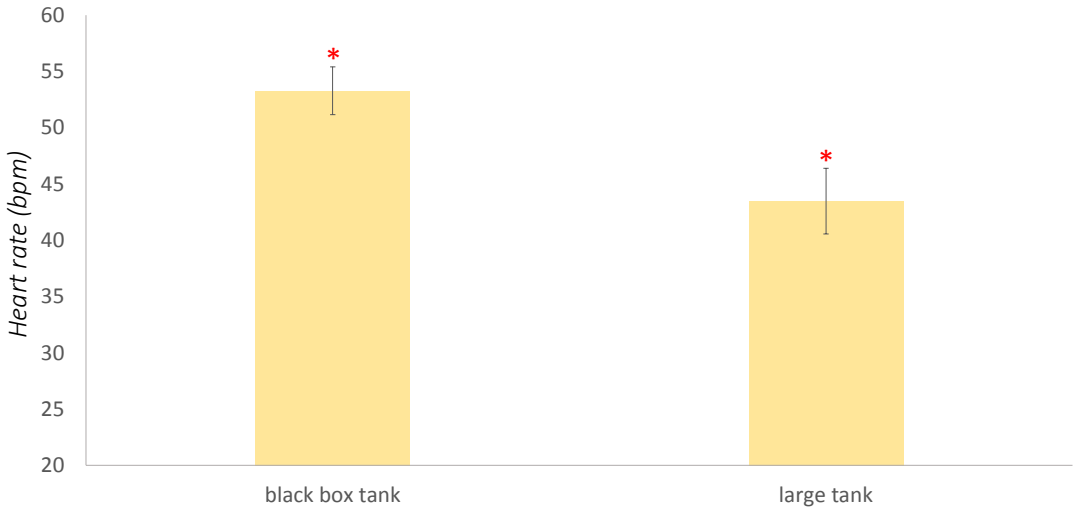

Fig. 8 Mean heart rate of the fish in the black box experimental tank two days after surgery compared to the mean heart rate of the fish in the large tanks three days after surgery. The Asterisk shows that heart rate differs significantly between the two, $p<0.05$. n=7 in both tanks.

Experiment 3

Fig. 9 shows the differences in ECG signal between three different positions of the implant in the same fish: b*)* the electrodes pointing sideways, *a)* two electrodes against the abdominal muscle tissue and *c)* one electrode against the abdominal muscle tissue. The signals from the three different positions of the implant differ both in overall amplitude and in the details of the waves. In position *a* the difference in amplitude between the P wave and QRS complex is larger compared to position *b* where a larger P and T wave can be seen. In position *c* the difference between the P wave and the QRS complex is smaller and no clear T wave can be seen. The best position detecting heart rate based on R-R intervals should be position *a* since this position shows the largest difference between the P wave and the QRS complex.

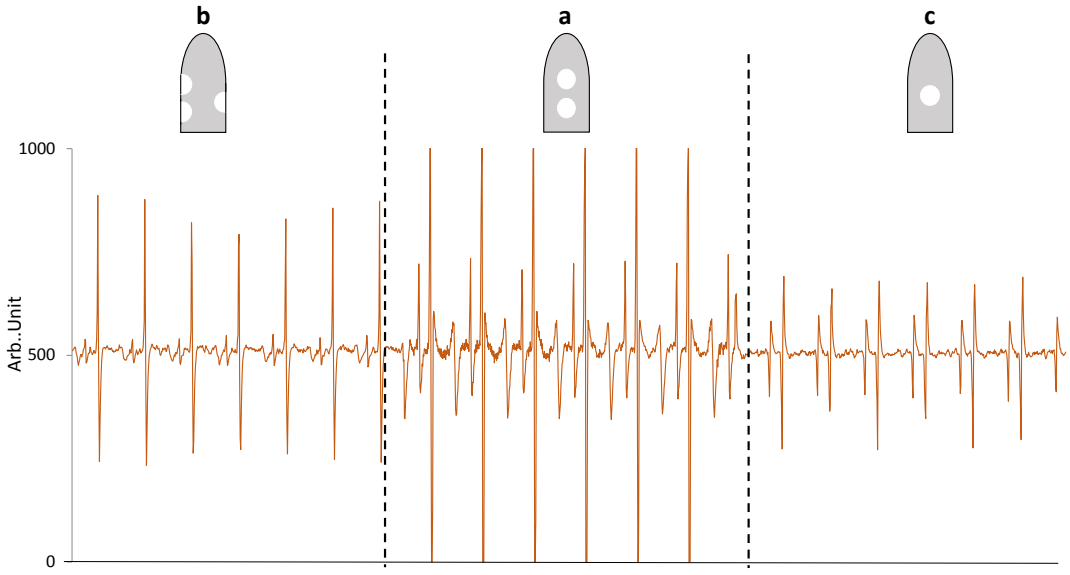

Fig. 9 ECG recordings of an anesthetized rainbow trout on the operating table. The graph shows the differences between positions *b*, *a* and c. All implants are positioned in the same area of the fish, but are rotated differently so that the electrode positioning differ. All three implant positions are displayed above the graph.

The next comparison was done between implant placement *b* and *e* (which is the same placement only another 20 mm posterior of the heart). **Fig. 10** shows that the strength of the signal is much weaker when the implant is positioned 20 mm further away from the heart.

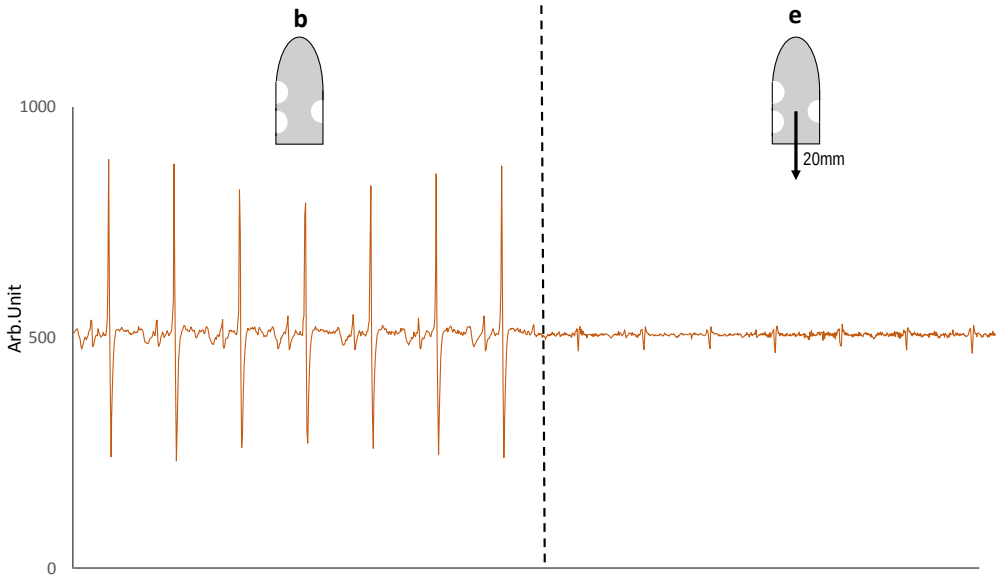

Fig. 10 ECG recordings of an anesthetized rainbow trout on the operating table. The Graph shows the effect in the ECG signal when positioning the implant in the proximity of the heart (b) compared to 20mm posterior of the heart (e). Both implant positions are shown above the graph.

The signal strength of *b* when positioned in an anesthetized fish on the operating table was compared with the same placement in the same fish when swimming freely in a large tank for 24 hours. **Fig 11.** shows a clear difference in signal strength between the two, as well as a much more instable signal with positioning *b* during the awake period, indicating that the movement of the fish affects the signal quality. Both of the placements gave clear QRS-peaks.

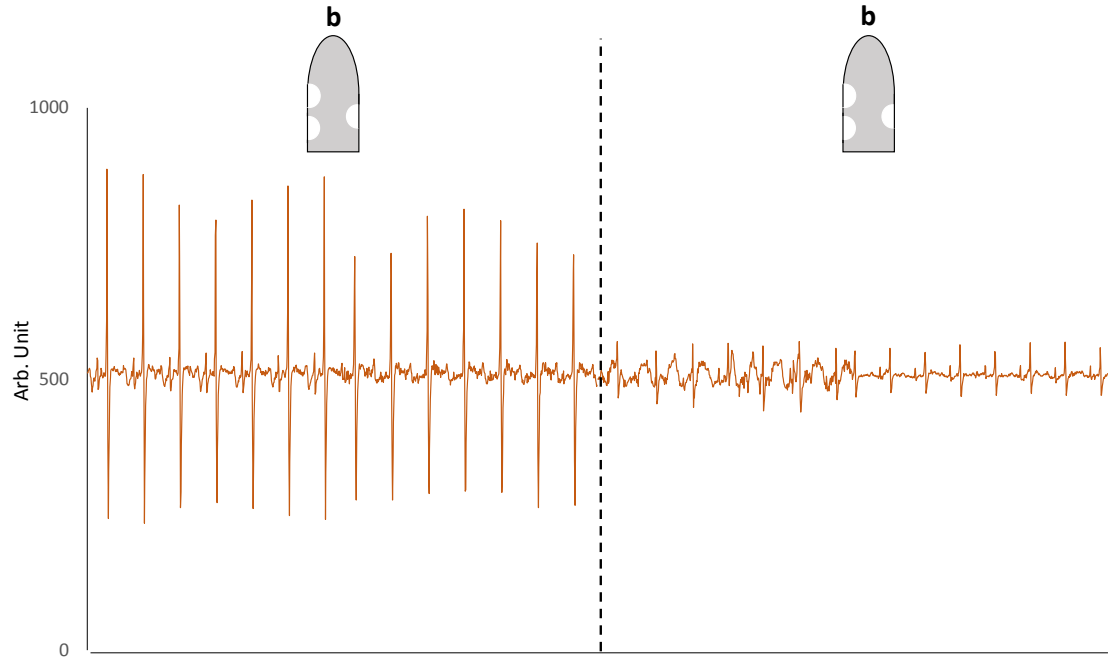

Fig. 11 ECG recordings of anesthetized rainbow trout on the operating table compared with swimming freely in tank. Both the implants were rotated to position *b*. The implant positions are shown above the graph.

Discussion

Stressor Response

Post-Surgery

Post-surgery heart rate for rainbow trout has been shown to be approximately 40 bpm one day succeeding surgery, decreasing with 5 to 10 bpm after one more day before stabilizing (Gräns et al., 2014). In Gräns et al. (2014), a 15 mm incision was made by the base of the pectoral fins which was stitched back up short thereafter to mimic a surgical procedure commonly used on rainbow trout. In our study, heart rate already seemed to stabilize one day after surgery. A further decreasing trend in heart rate was not found. In Gräns et al. (2014) the heart rate stabilized at approximately 30 to 35 bpm, while in our study heart rate was in between 40 and 45 bpm after three days. The results may differ due to the surgical procedures being different which might suggest that our surgical procedure is more invasive. On the other hand, the measured heart rates of the fish that underwent surgery without being treated with buprenorphine in Gräns et al. (2014) were presented in the study as being remarkably low. Other studies on rainbow trout such as that by Claireaux et al. (2005) for example, show much higher heart rate values. The size of the fish in the studies also differ, with our fish being somewhat smaller. All of these factors could contribute to the differences in heart rate between the studies.

Isolation, Interaction, Netting and Chasing

Moving the fish from their individual tanks to one shared tank gave a significant change in heart rate and also gave significantly higher heart rate than that measured after both the chasing and the netting of each fish. Chasing has been used as a stressor in several studies when investigating effects on heart rate (Barton et al., 1998; Iwama et al. 1999; Gräns et al., 2009a), but relocating fish from individual tanks to one shared tank has, to our knowledge, not previously been used in this way. Holding fish in a net for 60 seconds is also known a stressor (see Iwama et al., 1999). The netting did give significant changes in heart rate and also caused higher heart rate than the chasing, but not as high as after the grouping. The effects of the grouping also lasted longer than the effects of the netting. The results show that forcing a social interaction between the fish is potentially more stressful than chasing, along with the other tested stressors.

The results further show that when using the chasing protocol, which is regularly done in fish biology, the heart rate increase can be underestimated by almost 30% compared to the maximum heart rate following grouping. Measuring heart rate in fish during interaction seems to be a fairly new area of research and available peer reviewed literature is scarce. This is most likely due to the technical difficulties of performing such studies. This consequently leaves a possible area of research which could be pursued using implantable data loggers such as the DST milli-HRT.

Confinement

Our results show a significant difference between heart rate of the individuals in the confined black box tank and heart rate of the individuals isolated in the large tanks with the black box tank yielding higher heart rate. When it comes to research animals such as fish, based on our findings, it can be argued that some research equipment, such as the black box tank, may cause unwanted stress reactions. This is problematic for two reasons; one being the ethical aspect where the stress affects the welfare of the fish and the other problem is that research results may be unreliable due to the fish being stressed

prior to the research being done. This may hinder the fish from responding in a natural way (see Altimiras & Larsen, 2000). Confinement is a well know stress inducer which has been used as a standardized stressor in several previous studies (Campbell et al., 1994; Vijayan et al., 1997; Barton et al., 1998; Pottinger & Carrick, 1999). If we had based our findings solely on the black box results, the mean heart rate would have been higher compared to if we had based our findings solely on the large tank results.

Previously, it has been difficult, if not impossible to measure heart rate in fish while they swim freely in a larger tank. One form of measuring equipment, which has previously been used for recording blood flow as well as heart rate, requires attached electrode leads (Gräns et al. 2009a; Sandblom et al. 2010). This limits fish to more confined tanks so that the electrodes will stay attached. Another method which allows for less confinement is that of attaching electrodes to the bottom of a tank, and utilizing the conductivity of water to detect electric signals from the fish. This type of recording has been used in a few studies (Höjesjö, 1999; Altimiras & Larsen, 2000; Gräns et al., 2014), but it has been shown that the method gives a lot less clear signals when the fish are moving (Altimiras & Larsen, 2000). Though the confinement is not as great as that of the black box, the fish are still limited to a very small tank, which (from the results shown in this study) can still cause distress. Bio-logging will allow for the use of tanks even larger than the 500L used in this study, which goes to show the possibilities for this monitoring technique in comparison to other previously used techniques.

Implant placement

In experiment two, a lot of the measurements were unusable because of the amount of quality errors reported by the application software that comes with the implants. One possible and likely cause could have been the positioning of the implant. This was investigated in experiment three where the results showed a vast variety of signal quality depending on the placement of the implant. The best signal was observed when the electrodes of the implant were not contacting the muscle tissue or the fat tissue. This signal had a clear QRS-peak, which was a lot less clear with the other two placements. This is probably due to the other placements picking up more disturbances from other muscle groups. If these placements were tested in swimming fish, the likely disturbances observed with placements *a* and *c* would most likely be even more pronounced. This would have been problematic for measuring heart rate and might have been the reason why a lot of the measurements from experiment 2 came back unusable. Our findings also verify that the signal can be worsened when the fish is released back into the tank as the implant placement which gave the clearest signals gave a much weaker signal when tested in a swimming fish. Testing the impact which the movement of the fish has on the signal quality will have to be done in more detail with more individuals to be able to finalize the most optimal implant positioning. Experiment 3 also shows that the implants distance from the pericardium can have huge impacts on the signal strength and this can be a reason for the large signal variations in Experiment 2.

Conclusion and application in future research

While using a bio-logging system, several fish interacting with each other can be studied simultaneously under a very long time period. This shows an immense potential for application in a large amount of different research areas. Because of previous limits to monitoring physiological responses in fish, it has not been possible to monitor several fish interacting with each other. The high storage capacity of the implant in relation to the size and battery life time opens up for other research projects

such as monitoring heart rate and temperature during growth, smoltification or sexual maturation, all of which occur under a longer time period. In addition, being able to monitor heart rate while simultaneously providing fish with more space may also help to minimize unwanted effects of confinement stress, which in turn will give more accurate measurements and higher quality data.

This study, as a whole, might give an indication of how future studies of fish will take place. Implantable bio-logging systems such as the DST milli-HRT are part of a much needed step to be able to "move out of the box" and conduct successful ecophysiological studies in more natural environments. Being able to monitor several fish at once along with increasing the living space of the fish are both colossal contributions to fish physiology research because they bring researchers closer to being able to imitate the natural habitat of fish. This has been a lot more difficult prior to the introduction of more modern monitoring techniques such as bio-logging and bio-telemetry. It is worth stating though, that a lot more research is need in this field if more progress is to be made in the near future.

Application in biology didactics

The Swedish high school system is divided into different programs so that students in 9th grade can choose which orientation they would like to focus on throughout their final years of school. One such program is the natural science program, *naturvetenskapsprogrammet* in Swedish, which focuses on biology, chemistry, physics and mathematics while also covering all other mandatory courses.

A large part of the Swedish high school curriculum for biology is learning the scientific method. It is mandatory for students to show that they understand how to formulate and ask scientific questions, how to test these questions using experimental method and systematic observations, and critically analyze results from such tests. It is also important for the students to be able to set up arguments based on scientific findings and using scientific analysis. According to the biology curriculum, current research in biology should be incorporated into the education (Skolverket, 2011). This study, being scientific research, is directly applicable to biology didactics where it can be used as an example of working with scientific method. I would also argue that it strengthens my knowledge in scientific method and practical science significantly.

Another area of the curriculum is that of Biology's importance to the individual and to society as a whole. According to the Swedish National Agency for Education "studies in biology should give students enough insight to be able to understand and participate in current social debates and discuss ethical questions and standpoints" (Skolverket, 2011). The findings of this study actually show huge potential in areas such as sustainable fishing and sustainable fish farming where fish welfare has become a lot more common as a topic of debate.

The biology curriculum also consists of organism form and function, where human as well as animal physiology is included. From this area of the curriculum, students need to learn the different organ systems, their function and their connection to each other. They also need to understand the relationship between evolution, organism's functional build-up, and life processes (Skolverket, 2011). This study is directly related to animal physiology and requires knowledge in all these areas and is therefore also relevant to this area of the biology curriculum. Again, this also contributes to my knowledge of physiology, which in turn will be helpful during pedagogical practice.

In general, this study may be a bit complicated for most high school students to make sense of let alone replicate in any way. However, this is the case for all research, and must therefore be boiled down to its essential "take home lesson". The area of fish welfare requires more research, especially when it comes to commercial fishing and aquaculture. The ethical and environmental debates on the meat industry have often left fish on the side, but are included to a greater extent now then they have been previously. This study is partly related to this topic and can definitely be used as a basis to such a debate in a classroom environment.

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