Heart rate and swimming activity as indicators of post-surgical recovery time of Atlantic salmon (*Salmo salar*)

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Abstract

Background: Fish telemetry using electronic transmitter or data storage tags has become a common method for studying free-swimming fish both in the wild and in aquaculture. However, fish used in aquatic telemetry studies must be handled, anaesthetised and often subjected to surgical procedures to be equipped with tags, processes that will shift the fish from their normal physiological and behavioural states. In many projects, information is needed on when the fish has recovered after handling and tagging so that only the data recorded after the fish has fully recovered are used in analyses. We aimed to establish recovery times of adult Atlantic salmon (*Salmo salar*) after an intraperitoneal tagging procedure featuring handling, anaesthesia and surgery.

Results: Based on ECG and accelerometer data collected with telemetry from nine individual Atlantic salmon during the first period after tagging, we found that heart rate was initially elevated in all fish, and that it took an average of 4 days for heart rate to return to an assumed baseline level. Although activity levels assessed from acceleration appeared to be less affected by the tagging procedure, baseline levels were on average reached after 3.4 days for this parameter.

Conclusion: Our findings showed that the Atlantic salmon used in this study on average required 3-4 days of recovery after tagging before tag data could be considered valid. Moreover, the differences between recovery times for heart rate and activity imply that recovery time recommendations should be developed based on a combination of indicators and not just on e.g. behavioural observations.

Keywords: fish telemetry/biologging, Atlantic salmon, post-tagging recovery, heart rate, swimming activity

Background

Fish telemetry/biologging is a method for monitoring free-swimming fish where individual animals are equipped with electronic tags that often contain sensors for collecting data on the conditions within or near the fish (Cooke et al., 2011; Thorstad et al., 2013). Such tags may either be transmitter tags that transmit data wirelessly to the user (see Føre et al., 2011 for details on the structure of an electronic transmitter tag) or data storage/archival tags that store data in internal storage mediums that can only be accessed after the fish (and tag) has been recaptured (Thorstad et al., 2013). Irrespective of tag type, most studies using such methods aim to assess the status of wild fish in ecological settings (e.g. Welsh et al., 2013; Taylor et al., 2017) or address conservation and management issues (e.g. Cooke et al., 2004; Crossin et al., 2017). The interest in using this approach in aquaculture is also increasing, both because ongoing technological advances are rapidly expanding the possibilities (Hussey et al., 2015), and because new production philosophies such as Precision Fish Farming promote monitoring at an individual level (Føre et al., 2018a). Example uses of telemetry/biologging in aquaculture include studies to assess fish responses during critical operations such as crowding (e.g. Føre et al., 2018b) and transport (e.g. Brijs et al., 2018), and responses towards environmental variability such as temperature variations (e.g. Johansson et al., 2009).

In animal monitoring, it is essential to ensure that the animals observed are representative of the targeted population. For methods based on e.g. underwater cameras and echo-sounders/sonars which target sub-volumes, this can be achieved by adjusting instrument position and orientation to ensure observation of a representative subset of the population under investigation (Føre et al., 2018a). The situation is different when using telemetry as the fish selected for tagging must be representative both before and after the tags are deployed. Ideally, this means that the selection of fish should be truly random and representative, and that the tags do not influence physiology or behaviour in such a way that the tagged fish differ significantly from untagged fish (e.g. Wright et al., 2018). In addition, tagging procedures include several operations (i.e. handling, anaesthesia and surgical procedures) that may induce stress that in turn may lead to physiological and/or behavioural changes in the fish (Thoreau and Baras, 1997; Jepsen et al., 2001; Connors et al., 2002; Campbell et al., 2005; Thorstad et al., 2013). If such changes are chronic, the fish cannot be considered representative of the population and should be excluded from further analyses (Cooke et al., 2011). Conversely, if the changes are transient, the fish may be considered fully recovered once the response patterns return to those expected from an untagged fish. This means that tagged fish can be used in analyses if the data from the period immediately after tagging are excluded, but also raises the question: how can we define when a fish is properly recovered after a tagging procedure?

Jepsen et al. (2001) sought to identify the duration of post-surgery recovery for Chinook salmon (*Oncorhynchus tshawytscha*) by studying cortisol, glucose and lactate levels in their blood post-surgery (3 h, 24 h, 7 d, 14 d). The authors found that all these parameters were back to normal ranges 7 days post-surgery, with glucose and lactate normalising during the first 24 h, a recovery time resembling that seen in several studies (e.g. Martinelli et al., 1998; Bridger and Booth, 2003). Other studies have aimed to evaluate post-surgery recovery by comparing the behaviour of the tagged fish to their behaviour before surgery or in untagged cohabitant fish. This method has for instance been applied in laboratory experiments with tilapia (*Tilapia sp.)* who appeared fully recovered 24 h post-surgery after displaying lowered activity levels just after tagging (Thoreau and Baras, 1997).

Recovery after tagging may also be studied with sensor telemetry. The information conveyed by the tag must then reflect the state of the fish, and typical sensor values for unstressed fish should be available as a baseline for comparison. Previous studies using this approach include using heart rate tags to compare tagging methods for black cod (*Paranotothenia angustata* Campbell et al., 2005), and more recently to study post-surgery stress-responses (Brijs et al., 2019) and potential effects of antibiotics on post-surgical recovery (Hjelmstedt et al., 2020) in rainbow trout (*O. mykiss*). While the former study implied a rather wide window of recovery from surgical implantation (24 h – 20 days), the latter study suggests that rainbow trout on average are recovered 72-96 h after anaesthesia and surgery. Other sensor measurements that could potentially be used in this way includes swimming activity, as previous studies have identified links between activity and stress in salmon (Kolarevic et al., 2016; Føre et al., 2018).

Although Atlantic salmon (*Salmo salar*) is one of the fish species most frequently studied using telemetry, there is a lack of detailed quantitative information on the post-surgery recovery of this species. We therefore sought to identify the recovery time of Atlantic salmon after intraperitoneal tagging by studying individual measurements of both heart rate and activity in the period following surgical implantation. This resulted in a dataset with both physiological (heart rate) and behavioural (activity) indicators of tagging effects. The data were collected in a controlled experiment in tanks studying how stress responses in Atlantic salmon can be measured using state-of-the-art technology. The stress response part of this experiment is described in greater detail by Svendsen et al. (submitted for publication).

Materials and methods

Experimental site and fish

The experiments were conducted at the NINA Ims Research Station near Stavanger, Norway, between January and March 2019, using hatchery reared Atlantic salmon of the Aqua Gen strain (mean weight 2100 g). The experiment started on January 28th by stocking four square tanks (tank 1-4, 215 cm side, 122 m depth) with seven fish each. The fish were then allowed to habituate to the tanks until February 18th when three fish in each of tanks 1-4 were selected at random and equipped with tags, resulting in 12 tagged fish in total (three tagged fish and four untagged fish per tank). The tanks were set up with flow-through configuration, water being supplied from seawater inlets. Consequently, tank water temperature followed the ambient temperature in the fjord near the site, increasing from 3.9 to 5.0˚ C between the start and end of the experiment (March 15).

Biotelemetry/logging systems and surgical procedures

All 12 tagged fish were equipped with Data Storage Tags (DSTs) measuring heart rate (4 x DST milli-HRT, 39.5 x 13 mm, 11.8 g in air;4 x DST centi-HRT, 46 x 15 mm, 19 g ;4 x DST centi-HRT ACT, 46 x 15 mm, 19 g; Star Oddi Ltd.). Since all three tag types were from the same provider and contained the same type of heart rate sensor, they provided heart rate data sets that were comparable among tags. One tag type (DST centi-HRT ACT) also measured activity using an embedded tri-axial accelerometer. Two of the tagged fish in tanks 1 and 2 were also equipped with acoustic tags (A MP-9, 24.4 x 9 mm, 3.6 g; Thelma Biotel AS) that transmitted an activity proxy computed as the high pass filtered norm of all three axes in a tri-axial accelerometer (Kolarevic et al., 2016; Føre et al, 2017). In sum, the experiment thus produced 12 data sets on heart rate, and 8 data sets on swimming activity.

Each tag implantation was started by capturing a random fish from an experiment tank using a knotless dip net and immediately transferring it to an anaesthetic bath (Benzoak Vet, 70 mg/l) where the fish was kept until its equilibrium was lost and a state of anaesthesia was reached (average time 7.7 min). The fish was then carefully placed on a specialised surgical table with a v-shaped mid-section with its ventral side up. A hose circulating anaesthetic (Benzoak Vet, 35 mg/l) through the orobranchial cavity of the fish was inserted into its mouth and the head was covered by a moist cloth (Figure 1).

Figure 1: Fish in surgical table with anaesthetic circulation tube and head cover.

A 2-3 cm incision was made along the sagittal plane starting slightly more than one tag length (i.e. the length of the tag to be implanted) posterior from the transverse pericardial septum.

A finger was inserted through the incision to locate the transverse pericardial septum. While retaining the finger inside the peritoneal cavity for support, a needle was positioned in the skin just posterior to the transverse septum and slightly laterally from the sagittal plane. The finger was withdrawn, and a smooth plastic spoon inserted through the incision until it was just below the needle insertion point. The needle was then pushed through the peritoneal wall while simultaneously withdrawing the spoon to extract the needle out through the incision while protecting the viscera. One end of a suture threaded through the end of the tag was inserted into the tip of the needle. The needle was then withdrawn to pull the suture out through the needle's entry point. This procedure was then repeated on the other side of the sagittal plane. The tag was then inserted through the incision and anchored anteriorly in the peritoneal cavity using the suture and an (external) surgical knot. For the four fish also equipped with separate acoustic tags, the second tag was inserted into the peritoneal cavity through the same incision. Finally, the incision was closed using interrupted sutures. The fish was then transferred to a recovery tank with circulating seawater where it was kept until it regained consciousness, upon which it was transferred back into the tank it was collected from. The average duration of the entire procedure from being taken out of the anaesthesia bath until being released into their original tank was 7 min.

Timeline and experimental design

Since the present study focused on investigating the post-tagging recovery, the analyses only included data from the two weeks following tagging. To avoid inducing other stress effects that could disturb their recovery, the fish were sheltered from all potential stress factors except those necessary to feed and provide for the fish in this period.

None of the fish exhibited signs of adverse health after tagging or during the trials, and all fish were euthanised after the conclusion of the experiment. Post-humous pathology of all experimental fish (19 female, 23 male) revealed that about one third of the fish (8 f, 6 m) exhibited signs of sexual maturation through the experimental period. Although this appeared to have little direct impact on the fish in three of the tanks, the data from the fish in one of the tanks (tank 3) were excluded from the statistical data analyses due to perpetual inter-individual aggression between two matured males in that tank throughout the experimental period. This left nine fish tagged with DSTs measuring heart rate. Of these, six fish contained tags measuring activity. Two of these fish each contained both a DST and an acoustic tag measuring activity, providing a total of eight time-series of activity.

Data processing and statistics

Heart rate data were used as downloaded from the DSTs, only removing unrealistic outliers from the datasets. Activity data from the DST centi-HRT ACT tags were downloaded as raw acceleration values along all three axes, and then subjected to similar post processing as that used to compute the activity proxy in the A MP-9 acoustic transmitter tags to yield a comparable measure of activity between the two tag types.

Time-series of heart rate and activity were first decomposed into circadian, trend and random components using Loess smoothing (R function *stl{stats}*) because circadian and random variation has the potential to obscure trends in heart rate and activity resulting from post-surgery recovery. Long-term trend components were then analysed for a systematic change in heart rate or activity that could be indicative of a post-surgery recovery by firstly modelling the temporal relationship and then compartmentalizing this into pre- and post-recovery phases.

The relationship between the trend component of heart rate or activity (y) and time post-tagging (t) was modelled using an exponential decay model:

$$y(t) = y_a + (y_0 - y_f)e^{-\alpha t}$$

where α defines the rate of decay from y_0 (at time zero) to y_a, the model asymptote. Models were fitted with the nls function in R, using the self-starting asymptotic regression function SSasymp. Most trend components followed an exponentially decaying pattern, ensuring model convergence, but some included parts that were inconsistent with an exponential decay. Firstly, some tags (two heart-rate tags and four activity tags) showed a short initial post-surgery increase in registered values at the beginning of the experiment. Secondly, some tags (one heart rate and two activity tags) showed an increase in registered values after ≈ 5 d. To ensure model convergence, parts of the extracted trend components that were inconsistent with an exponential decline were removed prior to model fitting. One activity tag did not show an exponential decline with time and was thus not fitted with a model.

Identification of breakpoints between pre- and post-recovery phases was done on an individual basis. A recovery threshold was defined for each tag as the mean +2SD of the trend component values calculated from the final three days of the 14 day study period. Inspection of the tags showed that trend components were approaching asymptotes in the final three days, so it was reasonable to assume that values from these days represented post-recovery signature. Thresholds were established on an individual basis to allow for post-recovery heart rate or activity to change according to individual.

Results

Post-surgery recovery

Average heart rate declined post-surgery from ≈ 30 bmp to ≈ 21 bmp (Figure 2 A). Although some of the individuals carrying activity sensors also demonstrated a clear declining trend in the days following tagging, the trend was less clear across individuals (Figure 2 B). Both heart rate and activity displayed circadian variation, heart rate being greatest during daytime (Figure 2 A) whereas activity was greatest during night (Figure 2 B). This circadian variation was shown by all individuals, as revealed by the circadian components from the time series (Supplementary figure 1; Supplementary figure 2). There was generally greater activity during evening than during morning (Supplementary figure 2). Activities values registered by the acoustic tags were greater than those by the DSTs.

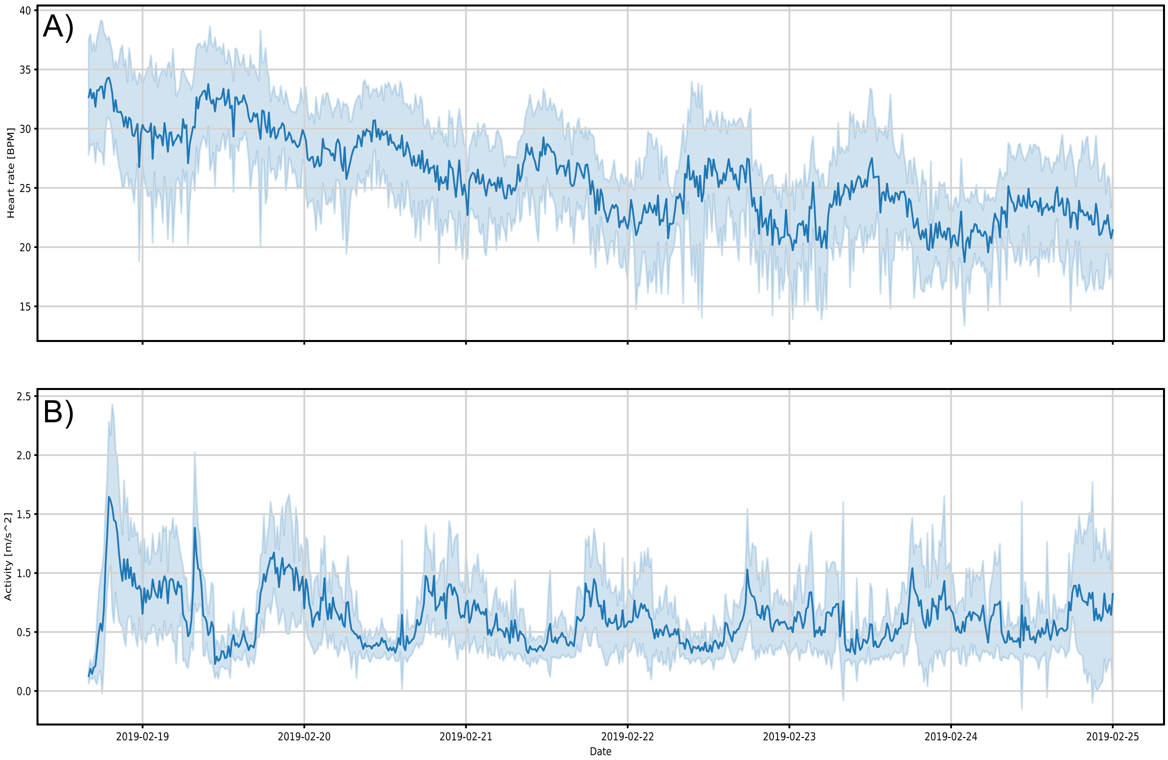

Figure 2: Average A) heart rate and B) activity values (blue line) from all fish in tanks 1, 2 and 4 (tank 3 was excluded because of aggressive behaviour between two males) for the first week post tagging. The light-blue envelope shows the range in heart rate values of all individuals.

The heart rate trend component showed a decline that could be modelled with an exponential decay function (Figure 3). The trend component however still showed considerable temporal variation, depending on the tagged individual. For example, the trend component for fish F4 showed a sharp decline during the first day after tagging, but this then fluctuated for the remainder of the two-week post-tagging period. The activity trend component also showed a pattern consistent with an exponential decay (Figure 4), except for one fish (fish F4) where an exponential decay model could not be fitted due to the activity trend component peaking ≈ 7 d after tagging.

Time to recovery (as defined by the location of the breakpoint between pre- and post-recovery phases) varied between individuals, and the metric used (heart rate or activity, Figure 3, Figure 4, Table 1). The mean heart rate threshold for recovery was 23.8 bmp (range = 21.2 – 26.0). The mean time to reach this threshold (i.e. breakpoint between pre-recovery and post-recovery) was 4.1 d (range = 1.3 – 5.8). The threshold for activity recovery was greater for the acoustic tags (mean = 0.42, range = 0.37 – 0.45) than the DSTs (mean = 0.28 – 0.31), reflecting the higher activity values registered by the acoustic tags. The mean time taken to reach the threshold was similar to that for the heart rate tags, but the range among individuals was greater (mean 3.4 d, range = 2.1 – 10.2). For the two individuals that were each tagged with two activity tags, the identified breakpoints between the parts of the time series classified as pre- and post-recovery depended on the tag: in both individuals, the threshold to reach post-recovery occurred later for the acoustic tag than the DST.

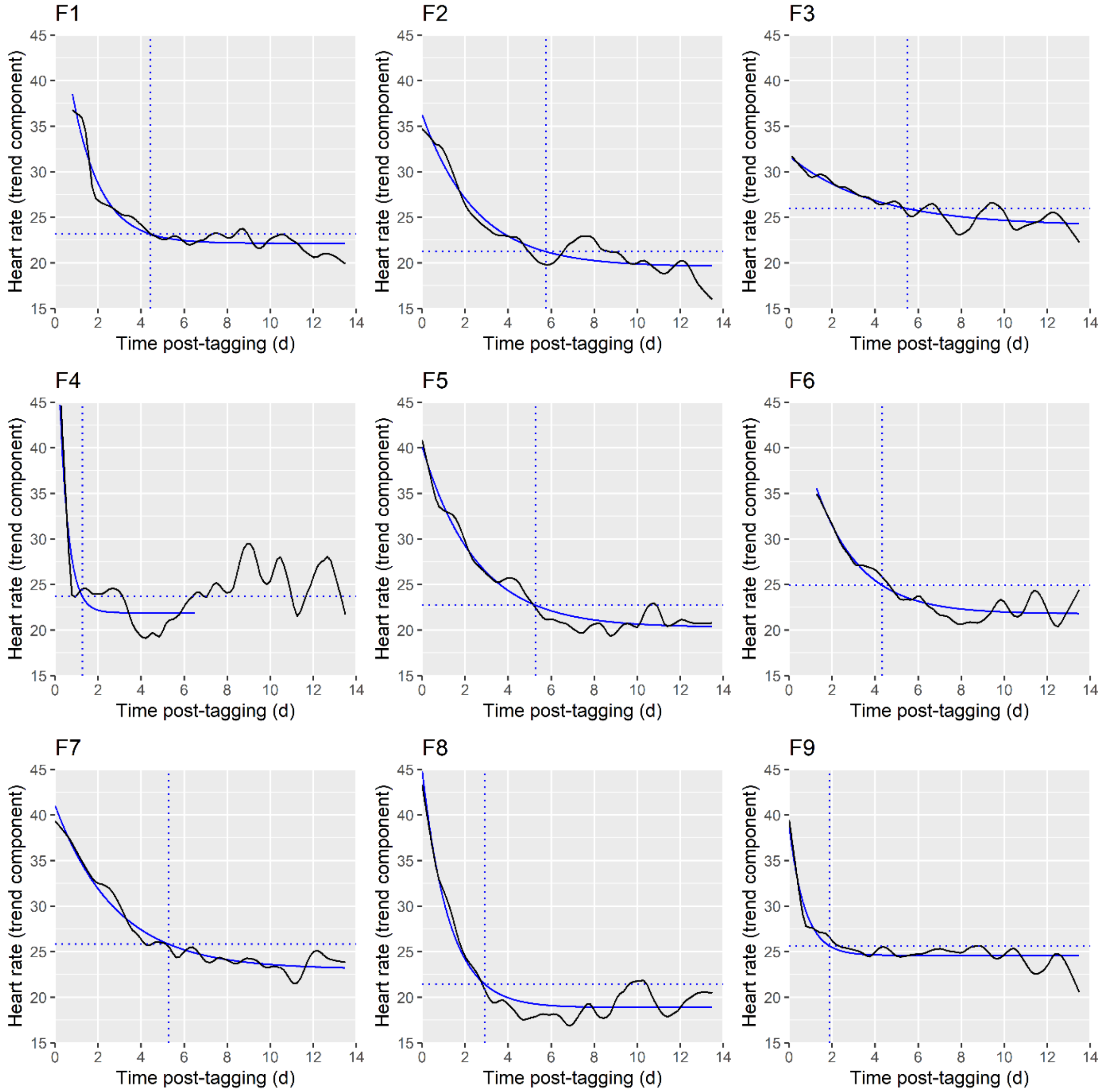

Figure 3: Long term trend in heart rate. The continuous black line shows the long-term trend component; the continuous blue line shows the fitted exponential decay model. The dashed horizontal line shows the threshold used to define a structural change; the dashed vertical line shows the breakpoint indicating when the structural change occurs between classified pre- and post-recovery phases.

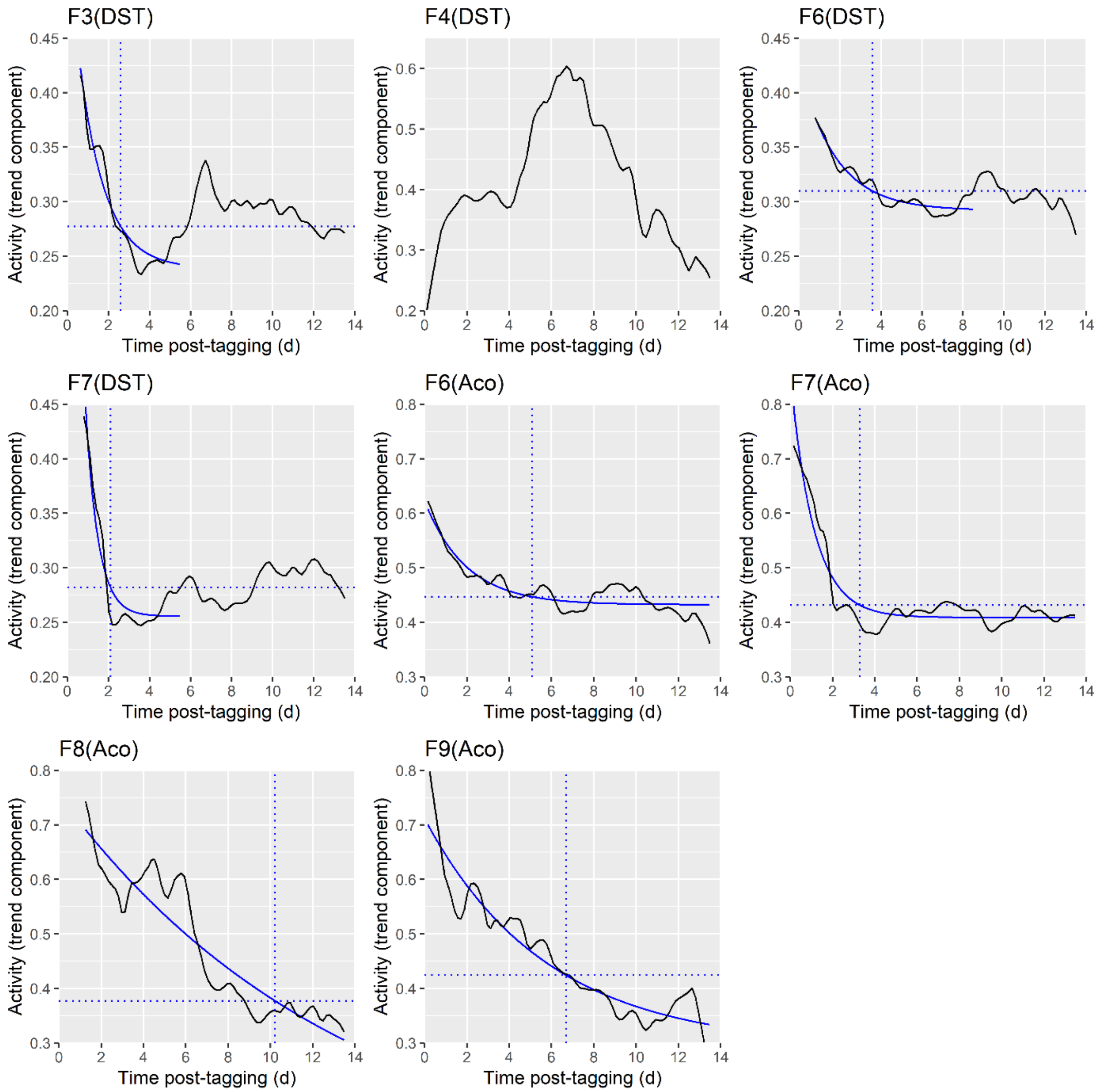

Figure 4: Long term trend in activity. The continuous black line shows the long-term trend component; the continuous blue line shows the fitted exponential decay model. The dashed horizontal line shows the threshold used to define a structural change; the dashed vertical line shows the breakpoint indicating when the structural change occurs between classified pre- and post-recovery phases. It was not possible to fit an exponential decay model to the long-term trend component of fish F4. Tag type (DST = Data Storage Tag; Aco = Acoustic tag) is shown in parentheses following the fish ID.

Although heart rate values just after anaesthesia and surgery varied more (31 to 45 bpm) than the recovery threshold (21.24-25.96 bpm, Table 1) and the post-recovery values, there was a clear declining trend for all tagged individuals. Heart rate values pre-recovery were significantly greater than those post-recovery (Wilcoxon signed rank test, V = 45, p = 0.001). Activity values pre-recovery were also significantly greater than those post-recovery, but the difference between pre- and post-recovery values was less (Wilcoxon signed rank test, V = 28, p = 0.007).

*Table 1: Recovery based on heart rate and activity sensors. Activity sensors with a * suffix indicate acoustic tags. NA indicates that the long-term component of the activity time-series did not follow an exponential decline.*

Tag ID	Heart rate recovery		Activity recovery	
	Threshold	Time (d)	Threshold	Time (d)
F1	23.17	4.42		
F2	21.24	5.75		
F3	25.96	5.50	0.28	2.60
F4	23.68	1.27	NA	NA
F5	22.71	5.29		
F6	24.90	4.33	0.31 0.45*	3.60 5.08*
F7	25.83	5.27	0.28 0.43*	2.10 3.30*
F8	21.43	2.92	0.38*	10.22*
F9	25.63	1.89	0.42*	6.69*
Mean	23.84	4.07	0.32	3.37

Discussion

The heart rate data suggest that the tagged Atlantic salmon in our study could only be considered fully recovered from the anaesthesia and surgical procedure of intraperitoneal tag implantation after at up to 6 days of recovery. This observation concurs with several previous studies that have reported similar lengths of recovery post-tagging (Martinelli et al., 1998; Jepsen et al., 2001; Bridger and Booth, 2003; Brijs et al., 2018; Brijs et al., 2019). Recovery results based on activity data varied more in threshold criteria and recovery times than heart rate, suggesting it might be a less consistent indicator of recovery between individuals. Moreover, both the temporal patterns and absolute values changed less for activity than heart rate between post-tagging and post-recovery periods, essentially implying a lower ratio between the baseline pattern (i.e. circadian variations) and the changes in activity caused by the tagging procedure. Together, these factors suggest that activity may be a less consistent indicator of post-tagging recovery than heart rate, and that heart rate might be a generally more sensitive indicator than activity, especially for post-tagging recovery.

Based on these results, we urge caution on using telemetry data collected shortly after anaesthesia and surgery without first ensuring that the fish are fully recovered. This evaluation should also be done carefully, as behavioural observations and comparison with untagged fish may not be sufficient to capture the full post-anaesthesia/surgery effects.

The surgical procedure used to implant the heart rate tags was much simpler than the procedure needed for multivariate implants recently used in rainbow trout by Brijs et al. (2019), but was more comprehensive and invasive than that used for conventional intraperitoneal tag placement. It is therefore possible that less complex surgical procedures would lead to shorter recovery times. However, it is probably reasonable to be conservative with respect to recovery times, especially if the data are to be used e.g. as a management tool in aquaculture applications or to evaluate stress effects on fish in conjunction with ecological studies. Using data from fish that are still recovering from post-anaesthesia/surgery effects in such applications could result in sub-optimal management decisions or erroneous conclusions that could have ramifications beyond the study itself.

The fish included in the analyses exhibited heart rates that gradually stabilized at daily means between 21and 26 bpm (daily variations between 15 and 30 bpm, similar to that observed by for A.

salmon of similar size at 4˚ C by Lucas, 1994). Due to the similarities across tanks and individuals this range in heart rate may be typical for Atlantic salmon of this size and with the prevailing temperatures. Moreover, all individuals in tanks 1, 2 and 4 had similar circadian rhythms (higher heart rates during daytime than at night) and gradual post-surgery declines in mean heart rate (from more than 30 bpm after surgery, to 21-26 bpm after up to six days). This implies a regularity across individuals that increases the likelihood that heart rate may function as a consistent stress indicator in Atlantic salmon that may be used to assess fish recovery after tagging. The tagged fish in tank 3 were excluded from the study due to inter-individual aggression, which was also seen in that the measured heart rates differed for these fish both in individual and aggregate values (Supplementary figure 3). Although these fish showed signs of circadian variation in heart rate, the mean value did not appear to decline over the days following tagging. This may indicate that the stress induced by the aggression between the two males in this tank overrode the stress response caused by handling, anaesthesia and surgery. A potential interpretation of this is that the stress impact of tag implantation is moderate compared with the stress induced by other external events, assuming that the fish surgery is conducted according to recommended practices.

Although this study underlines the importance of critical evaluation with regards to recovery from anaesthesia and surgery when using telemetry, the data collected also highlight the importance of telemetry as a method for studying free swimming fish. All tagged fish appeared to eventually recover from the surgical procedure, and posthumous pathology revealed no inflammations or other apparent morphological signs of reduced welfare due to the surgical procedures. Even though the low water temperatures during the experiment may have led to handling and surgery having less impact on the fish, the tagging procedure used here was more complex than conventional intraperitoneal tagging. It is thus reasonable to conclude that fish carrying telemetry tags can be considered representative members of the group they were selected from once they are fully recovered from anaesthesia and surgery, provided that they were a representative selection to begin with. However, this also requires that the recommendations on ratio between tag size and fish size are not exceeded (which was not a challenge in our case working with adult salmon).

Future research and potential technological improvements

Since this study only focused on Atlantic salmon, exposed to one set of environmental conditions, it is difficult to assess if these concerns are also relevant for other species, and/or fish under different conditions. Similar studies on rainbow trout using the same tag type found that they recovered a little faster (on average 72-96 h) than the Atlantic salmon in the present study (Brijs et al., 2019). Moreover, wound healing in Atlantic salmon is known to depend on temperature (Jensen et al., 2015), suggesting that the low water temperatures in the present study may have contributed to longer recovery periods. These elements suggest that species specific effects or differences in external environmental conditions are important to consider when studying recovery times. Future studies on the relationship between heart rate and post anaesthesia/surgery recovery time for other species of interest and at higher temperatures should therefore be conducted to obtain a more complete picture of this relationship.

In the present experiment, the fish were kept in small tanks in groups. Future studies addressing post-tagging effects should aim to make similar measurements at larger spatial scales, with a larger number of tagged fish. This would enable the detection of eventual changes in response to scale effects and social/inter-individual effects arising due to group dynamics. To increase the realism of such a study, it could be done in fish cages in the marine environment, perhaps first by using meso-scale size cages containing fewer fish but at the same density as in a commercial cage, and then moving to full-scale studies to complete the chain of arguments from lab to industrial scale.

Conclusion

The main conclusion from this study is that the Atlantic salmon in these experiments needed 3-4 days of recovery on average after anaesthesia and surgery before their heart rates returned to levels that could be assumed to be baseline resting values. Moreover, although observation of behaviour and/or activity may alone be insufficient to assess that the fish has physiologically recovered, activity measurements indicated similar recovery periods to those based on heart rate, although there was a longer maximum period of 10 days for one individual based on activity. We therefore urge caution when using data collected shortly after surgery and anaesthesia in studies using biologging/telemetry tags. The duration of this period may however vary among species or with a range of external and internal factors.

Declarations

Ethics approval and consent to participate

All fish handling and surgery were made in compliance with the Norwegian animal welfare act and were approved by the Norwegian Animal Research Authority (permit no. 18/18431).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests in this section.

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Authors' contributions

MF manages the funding project, participated in the planning and execution of the experiments, and did the main job in preparing the manuscript. ES participated in the experiments, prepared the data, and was central in analysing the data. FØ was central in planning the experiments and conducted the surgery to equip the fish with the tags (together with AG). AG programmed the heart rate loggers, conducted the surgery to equip the fish with the tags (together with FØ) and contributed in the data analyses. JAA participated in the planning of the experiments and data analyses. BF was the main person behind the planning and execution of the experiment and participated in the experiment and data analyses. RH was responsible for the statistical analyses and contributed in the general processing of data. IU had a key role in planning the experiments and had the original idea behind the study resulting in this article. All authors have contributed in writing the manuscript and approved it before submission.

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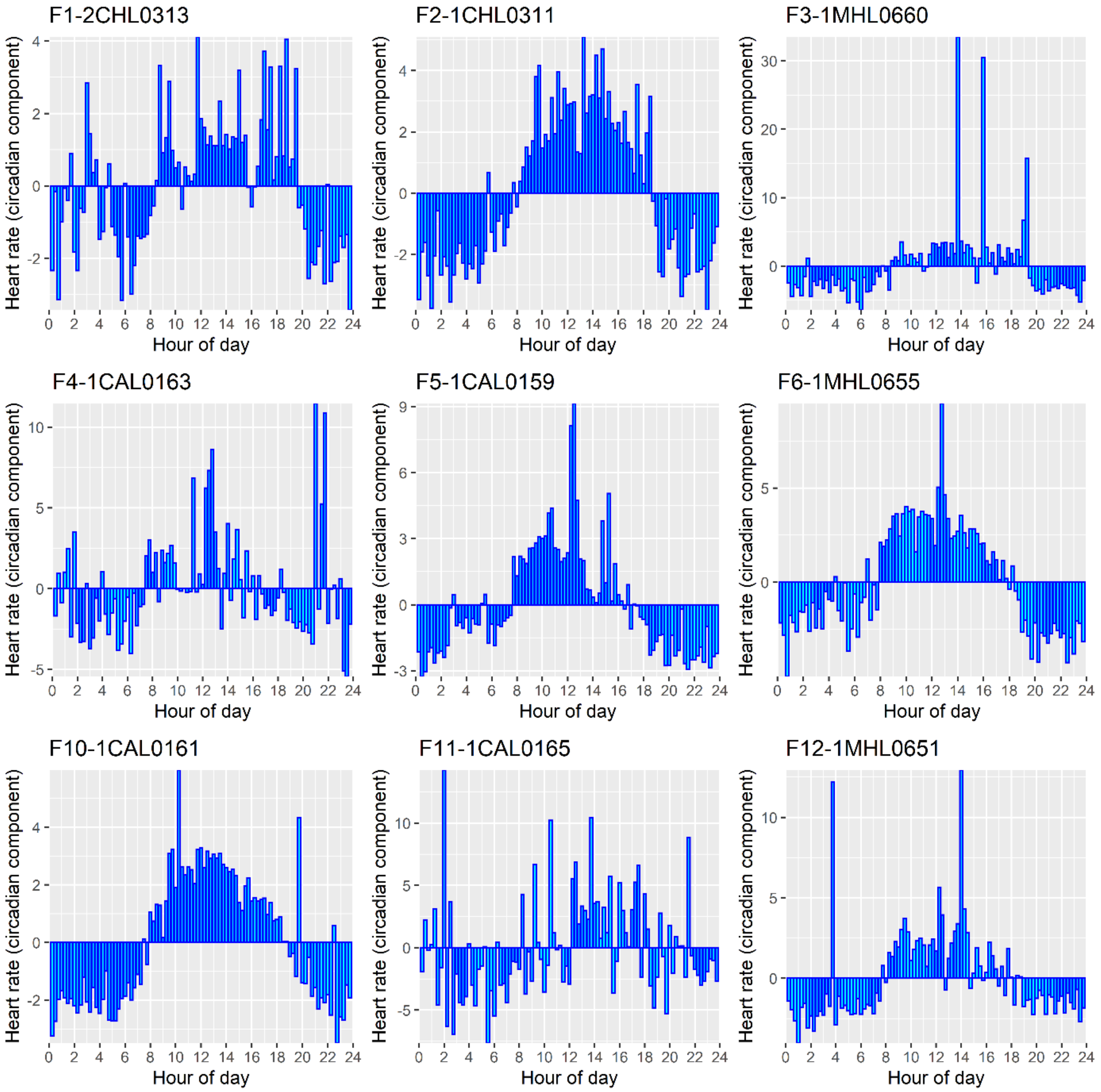
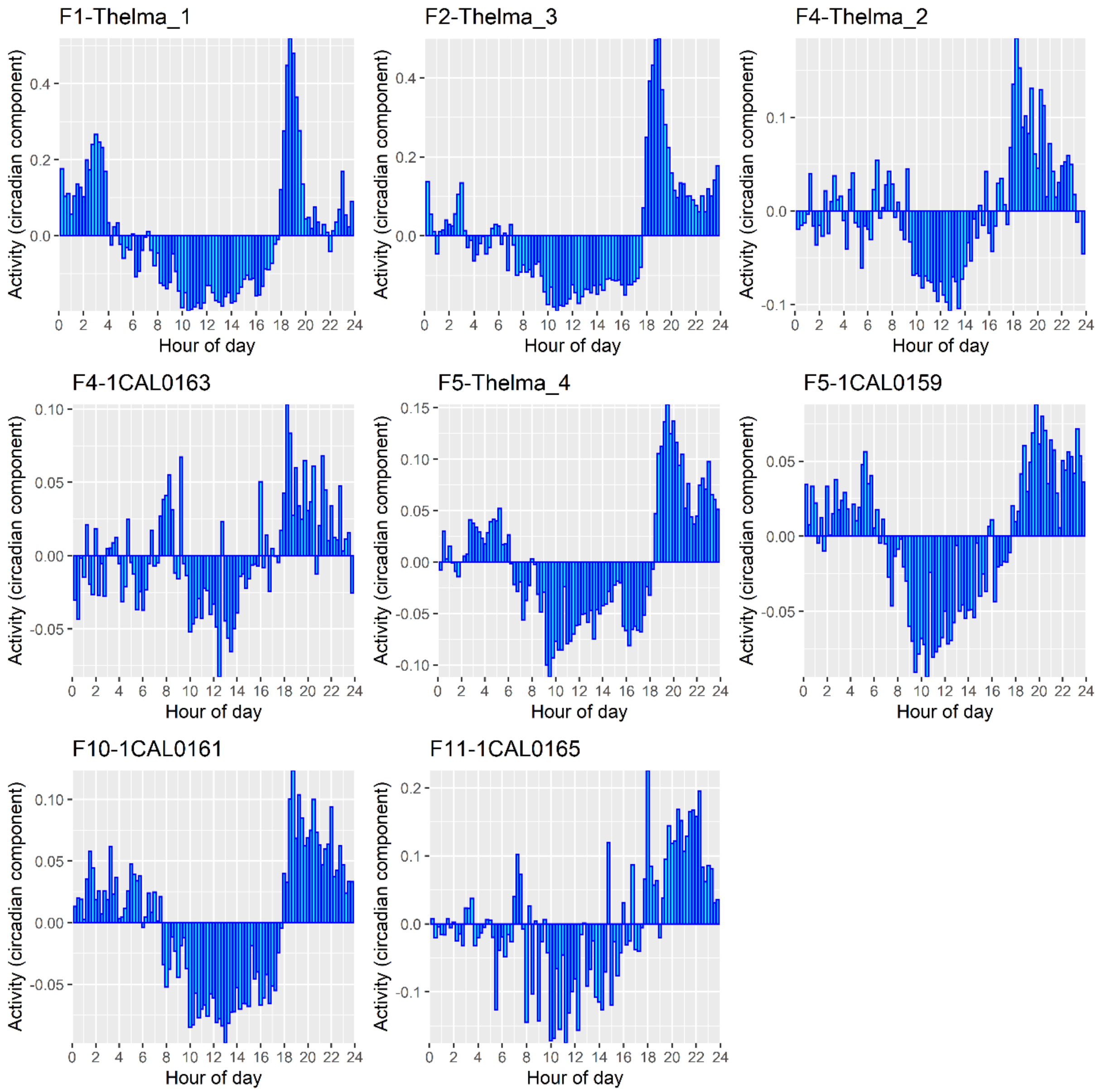
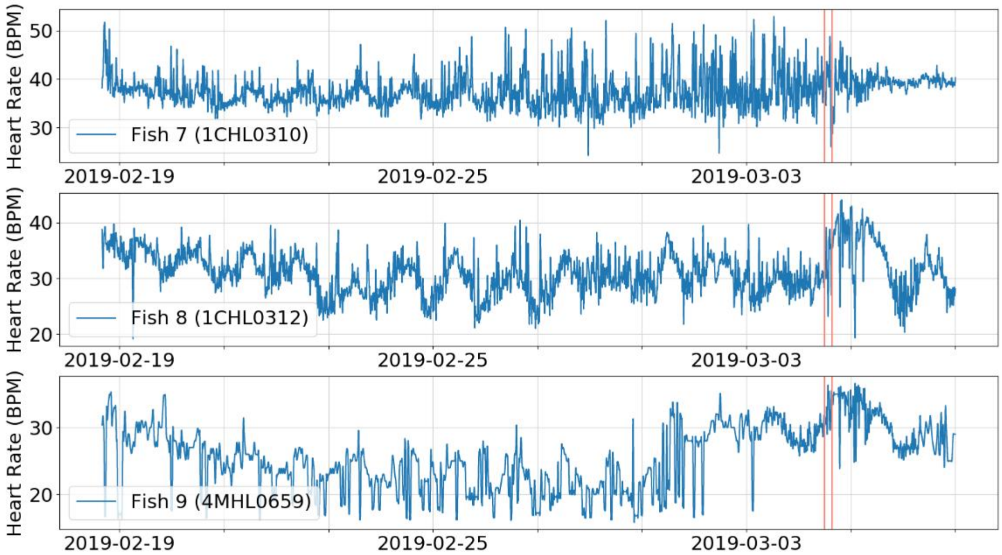
Supplementary Material:



Supplementary figure 1. Circadian component of heart rate.



Supplementary figure 2. Circadian component of activity.



Supplementary figure 3: Heart rate plots for the three tagged individuals in tank 3 which contained the two fighting males