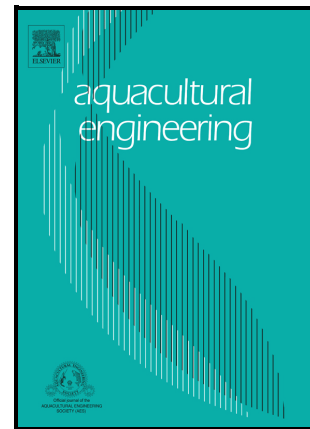


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# Behavioural response of farmed Atlantic salmon (*Salmo salar* L.) to artificial underwater lights: wavelet analysis of acoustic telemetry data

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## Abstract

Biological, environmental, economic and ethical issues become increasingly pertinent as the scale of the aquaculture industry expands. This study used acoustic telemetry data and wavelet analysis to investigate behavioural patterns of Atlantic salmon when exposed to artificial underwater lights in fully stocked production cages located on the Norwegian coast. Using acoustic depth sensor tags, time series of depth registrations were gathered from 21 individual salmon distributed over three cages during a five-month experimental period. Underwater lights, normally used to suppress pre-harvest sexual maturation amongst Atlantic salmon, were installed at eight-meter depth and switched on in the middle of the experimental period. Swimming depth registrations initially showed a typical diurnal swimming behaviour, manifested by registrations generally in deeper waters during day-time than during night-time. The diurnal swimming behaviour abruptly ceased after the onset of lights. The change in swimming behaviour was detected by wavelet analysis and coincided with the introduction of underwater lights. Results from this study demonstrate the utility of wavelet analysis as a timely surveillance tool when investigating behavioural patterns of a periodic nature in fish, and specifically the individual response of farmed salmon to artificial lighting in a genuine industrial setting.

**Keywords:** Acoustic telemetry; Fish behaviour; Finfish aquaculture; *Salmo salar*; Underwater light; Wavelet analysis

## 1. Introduction

Observing fish and their movements in the sea cages forms a vital part of daily salmon farming husbandry. Good animal welfare is required by law for fish farmers in Norway. Biological, economic, environmental, and ethical issues become increasingly relevant as the scale of the aquaculture industry expands. Fish behaviour is routinely incorporated as one of the key welfare indicators during production (Martins *et al.* 2012; Macaulay *et al.* 2020). Behavioural indicators of poor fish welfare may include changes in foraging behaviour, aggression, and changes in diurnal depth cycles (Conte, 2004; Oppedal *et al.* 2011; Martins *et al.* 2012). By observing swimming depth over time under normal conditions, one can establish baseline periodic behaviours of swimming depth. This allows us to detect deviations from normal behaviour, which can be used as an early warning of potentially adverse conditions in the fish farm, allowing time to initiate closer investigations and implement mitigating measures (Oppedal *et al.* 2011; Hvas *et al.* 2020). However, such changes can be challenging to detect in full-scale production cages given the size of the sea cages and the number of fish in modern-day aquaculture. In Atlantic salmon (*Salmo salar* L.) farming, typical tasks include manual sightings and sampling of fish for health and welfare assessment through monitoring of surface activity and manually counting sea lice. These surveillance tools are based on direct visual inspection, relying on *in situ* observation of the fish. While visual inspection can serve as a straightforward and reliable method for gathering relevant data on fish behaviour and welfare, it is labour-intensive and depends on physical presence at the farm site over extended periods of time. Manual sighting of fish behaviour is also limited to activity close to the surface. Consequently, the industry has become increasingly reliant on underwater cameras which provide remote observations of fish, e.g. during feeding (Føre *et al.* 2018a). However, using manual observations either in person or through cameras to track movements of fish stocked at high densities in large volumes of water is difficult and has limitations related to light conditions, visibility, and recognition of individual fish.

Light regimes have been shown to have a significant influence on sexual maturation in salmonids (Thorpe 1994; Taranger *et al.* 1998; 1999; Leclercq *et al.* 2011). To prevent sexual maturation during the production cycle of farmed Atlantic salmon, artificial underwater lights are often installed in cages during the darkest months of the year. Artificial lights have also been shown to enhance appetite and growth rate (Endal *et al.* 2000) and are thus adopted as a common farming practice throughout the industry.

The use of acoustic fish telemetry to obtain individual observations of farmed fish has shown promising potential over different studies throughout the last decades. Studies examining behaviour during feeding (Alfredsen *et al.* 2007; Føre *et al.* 2011), stress-inducing incidents such as crowding before sea lice treatments for Atlantic salmon (Føre *et al.* 2018b), and the assessment of behavioural responses related to temperature preference (Oppedal *et al.* 2007), cage distribution patterns (Leclercq *et al.* 2018), swimming depth (Muñoz *et al.* 2020), heart rate (Svendsen *et al.* 2021) and swimming

activity (Rillahan *et al.* 2011; Kolarevic *et al.* 2016) over a range of species have all contributed to new knowledge within their respective areas. Although limited by the number of individuals that can be tagged simultaneously, acoustic tagging studies generate vast amounts of data over time. This requires proper analytical techniques and tools capable of handling and assisting in the interpretation of such data, at both population and individual levels (Føre *et al.* 2018a; Brownscombe *et al.* 2019). The behavioural patterns of fish may vary from fluctuations of irregular and transient nature to more structured and recurring patterns. Behavioural changes, both sudden and more gradual, can be caused by changes in environmental conditions or changes in the health or physiological state of the fish (Martins *et al.* 2012), and are thus of interest to the farmers. Analysing such trends over time and identifying alterations in behaviour can therefore aid in the understanding of fish responses to environmental and anthropogenic changes.

When analysing the frequency content (periodic behaviour) of a time series, it is common to decompose the signal into a weighted sum of different periodic functions. The importance of each frequency can then be compared and assessed through these weights (Cazelles *et al.* 2008; Bjørnstad, 2018). The most famous type of periodic analysis is Fourier analysis, where the signal is represented as a sum of sine and cosine basis functions, with different frequencies. However, traditional Fourier analysis assumes that the periodicity is constant in time, and thus cannot be used to study changes in trends. On the other hand, wavelet basis functions are localised functions. Wavelet analysis allows for studying local variations in frequency, i.e. how the periodic signals vary over time (Torrence and Compo, 1998; Grenfell *et al.* 2001; Bjørnstad, 2018), and can thus be applied to detect change-points in periodic behavioural trends, for example in the depth usage of fish in a sea cage. In ecology, time series are often non-stationary, and hence there are many examples where wavelet analysis has been used to identify changes in periodic signals with ecological applications (Cazelles *et al.* 2008; Rouyer *et al.* 2008). Wavelet analysis has been used to identify changes in epidemic measles outbreaks in London after vaccination, finding for example an increase in the epidemic period after vaccination (Grenfell *et al.* 2001). Wavelet analysis has also been used to study transient periodic grouse abundance (Cazelles *et al.* 2008) and changes in periodicity in Atlantic sea birds (Jenouvrier *et al.* 2005). By studying three Atlantic sea bird species and analysing the fluctuations in the populations, a change was identified, suggesting a regime shift in the environmental conditions (Jenouvrier *et al.* 2005). Wavelet analysis can also be used to analyse spatial synchrony in time and has been used to identify for example travelling waves (outbreaks shifting from one location to neighbouring locations) in larch budmoth population densities (Johnson *et al.* 2004) and measles dynamics (Grenfell *et al.* 2001).

The objectives of the present study were to further explore the feasibility of using acoustic telemetry as a monitoring system for fish behaviour in full scale production units in aquaculture. Furthermore, we apply a novel analytical approach to such timeseries data to identify what we believe to be normal

diurnal cycles in swimming depth for farmed Atlantic salmon and sudden abruptions in such cycles. The application of wavelet analysis proved efficient in identifying abrupt shifts in swimming behaviour, from diurnal cycles in depth registrations to a state where the periodic behaviour disappeared. This shift in swimming behaviour seemed instantly induced by the onset of underwater lights in the production cages.

## 2. Materials and methods

### 2.1 Location and experimental facilities

The study took place from November 2016 throughout March 2017 at the commercial salmon farm site Kråkholmen, Norway (64° 36.165' N; 10° 51.220' E, Bjørøya AS). The farm layout, cage type, and the experimental cages' location within the farm are indicated in Figure 1. The farm was arranged as a single row of cages from south to north, with a feed barge moored to the west of the cages, as shown in Figure 1. Three out of a total of nine full-scale production cages, hereafter denoted as cages A, B and C, were chosen for the experiment. The cages had a cylindrical shape with a circumference of 160 m and 20 m depth and were appended by a bottom cone with a centre depth of 25 m (Polarcirkel plastic pen, AKVA group ASA, [www.akvagroup.com](http://www.akvagroup.com)). The farmed fish were of the AquaGen strain and stocked to the sea cages from Namdal settefisk in medio October 2016 weighing 100-110 g in stocking densities ranging from 150 000 – 171 000 individuals for the experimental cages.

Measurements of temperature, salinity, and oxygen depth profiles (CTD-O) were performed by using a SAIV SD204 probe ([www.saivas.no](http://www.saivas.no)) at approximately two-week intervals, from a fixed position near the farm's feed barge (Figure 1) by lowering it from the surface down to 35 meters of depth, at approximately 1 meter per second.

### 2.2 Experimental setup

The cages selected for the experiment were operated in the same manner as the rest of the farm cages. This included feeding with pneumatic feeders during working hours (08:00-16:00), daily inspections, mortality removal, and other common husbandry tasks.

#### 2.2.1 Underwater lights

Underwater lights were installed in all cages and switched on at approximately the same time of day (11:00 - 12:00) on the 20<sup>th</sup> of December in cage A, and on the 22<sup>nd</sup> of December in cages B and C. The underwater lights were switched on continuously throughout the research period, which lasted from 1<sup>st</sup> November 2016 to 2<sup>nd</sup> February 2017 (see 2.4 Data collection). The setup of lights comprised two rows of five lamps, where each row consisted of three SubLite Integra 1000W and two BlueLED 400W lamps (AKVA Group ASA, Norway). The lamps were interspersed at nine-meter intervals along the rows to achieve a relatively homogenous light distribution. The two lamp rows were

submerged to approximately eight-meter depth and spanned across the sea cage directly from west to east, as shown in Figure 1.

## 2.3 Acoustic telemetry

### 2.3.1 *Fish sampling and surgical protocol*

Fish were sampled from their respective cage using a hoop net down to approximately ten-meter depth, and the samples were taken in small batches (five to ten individuals) to minimise stress and handling time. A total of 23 fish were sampled and implanted with acoustic transmitters. The tagged fish were distributed over the three cages as follows: Cage A: N = 7, L =  $23.3 \pm 0.9$ ; Cage B: N = 8, L =  $25.4 \pm 0.4$ ; Cage C: N = 8, L =  $25.6 \pm 0.8$ , where N and L indicate the number of fish and the fish' total length in centimetres (mean  $\pm$  standard deviation), respectively.

Surgical procedures followed a well-documented protocol of anaesthesia and transmitter implantation in Atlantic salmon (Urke *et al.* 2013). Fish were anaesthetised using 60 mg L<sup>-1</sup> tricaine methanesulphonate (MS-222; Finquel® Argent Chemical Laboratories, Redmond, WA 98052). After reaching full anaesthesia, the total length of the fish was measured to the nearest centimetre, and the fish was placed on a v-shaped surgical table. A silicone hose attached to a circulating pump fed a continuous flow of anaesthetic fluid over the gills during the entire surgical procedure (20-30 mg L<sup>-1</sup> MS-222). An incision (10-12 mm) was placed slightly offset of the ventral line, about 2 cm behind the pectoral fins. Acoustic transmitters were rinsed in ethanol, left to air-dry, and subsequently inserted intraperitoneally into the fish. The incision was closed with three interrupted double surgical knots using a non-absorbing 4/0 monofilament suture (www.resorba.com) and sealed with a tissue adhesive (monomeric n-butyl-2-cyanoacrylate, Histoacryl®, www.bbraun.com).

Following the surgical procedure, fish were transferred to 400-litre recovery tanks continuously refreshed with seawater where they were kept until reaching full consciousness (2-6 minutes), and after that carefully released into their respective cages. All personnel involved in the surgical procedure had long experience with this procedure, and the study was approved by the Norwegian Animal Research Authority (FOTS ID 7835).

### 2.3.2 *Transmitters and receivers*

Acoustic transmitters with an integrated hydrostatic pressure sensor were selected for this study (ADT-MP9, Thelma Biotel AS, www.thelmabiotel.com). These were programmed to transmit data (fish ID + sensor value) on average every 90 s, with a random variation of  $\pm 30$  s to reduce the probability of repeated code collisions in the same frequency band. The tags had a cylindrical shape with a diameter, length, weight in water, and operating life of 9 mm, 26.5 mm, 2.7 g, and 375 days, respectively. Pressure readings provided information on swimming depth and were used to analyse behavioural patterns related to the fish's vertical position in the water column. The onboard temperature-compensated pressure sensor had an accuracy of  $\pm 150$  Pa, and the actual depth

measurements were received from each transmitter as 8-bit binary values with a linear correspondence to depths in the range 0-51 m, giving a depth resolution of 0.2 m.

Three acoustic receivers (TBR700 RT, Thelma Biotel AS) were deployed and submerged to 3 m depth in each cage (Figure 1). Each cage was assigned separate carrier frequencies (Cage A: 68 kHz, Cage B: 70 kHz, Cage C: 69 kHz) to reduce transmitter interference between the cages and increase the overall data acquisition capacity of the telemetry system.

The receivers were equally spaced at fixed positions at 3 m depth along the floating collar of the cage perimeter. Deploying multiple receivers in each cage served as a safeguard for persistent data collection, providing data redundancy in case of receiver malfunction or unforeseen attenuation/noise problems within the cage's acoustic environment.

#### 2.4 Data collection

Data were downloaded every 30 days in association with battery changes for the receivers. The collection period was initially planned to last from 1<sup>st</sup> November 2016 until 30<sup>th</sup> March 2017. However, practical problems with battery supplies between the 2<sup>nd</sup> and 5<sup>th</sup> February introduced gaps of variable length in the time series, until the receivers again were operational from 16<sup>th</sup> February. To preserve the consistency of the data material employed in the wavelet analysis, the period from 1<sup>st</sup> November 2016 to 2<sup>nd</sup> February 2017 was selected as the focus of the analysis. However, data from the entire period until 30<sup>th</sup> March 2017 are included in the appendix.

Data from two of the 23 transmitters were lost during the study period. One transmitter malfunctioned and failed to transmit any signals (Cage A) and one transmitted stationary depth registrations shortly after tagging (Cage C), suggesting either tag repulsion, sensor failure, or mortality. These two transmitters were excluded from the analysis.

#### 2.5 Statistical analysis

All analyses were performed using R software v.4.0.3 (R Core Team (2020)). Water temperature, salinity, and oxygen data were processed and plotted in R using the R-package *ggplot2*. Times of sunrise and sunset were obtained from the R-package *suncalc* to define day and night and plotted together with the time-series of depth registrations for all individuals. Depth registrations were also plotted over time using the R-package *ggplot2*.

To investigate periodic behaviour in the time-series, wavelet analysis was applied to the data. The R-package *Rwave* was used to perform the analysis, using the Morlet wavelet function. Because the time-interval between samples of acoustic telemetry data is generally irregular, the data were aggregated to a coarser time resolution of one hour, by averaging all samples received within each hour for each individual fish. If there were no observations within the hour, linear interpolation was applied to fill in the missing values.

For each frequency, the modulus at each time point was computed for all individual fish and compared on individual levels. A comparison of the moduli was done to identify and compare the strength of different periodic signals in the time-series. Phase coherence for the dominant period was examined by visually comparing the phase angle for all pairs of individuals within the same cage over time.

### 3. Results

#### 3.1 Environmental conditions

The CTD-O profiles showed a gradual drop in water temperatures from 9.3 to 5.7 C°, while salinity was relatively stable and mostly within the range of 30 - 33 ‰. No clear thermoclines or haloclines were present over the experimental period. Oxygen levels ranged from 8.1 - 10.2 mg L<sup>-1</sup> (Figure 2).

#### 3.2 Swimming depth registrations

Altogether 783 436 depth registrations were collected from 1<sup>st</sup> November 2016 to 2<sup>nd</sup> February 2017 (Cage A: 264 440, Cage B: 284 395 and Cage C: 234 601) with total detections per individual fish in the range 33 074 – 41 458. This corresponds to an average of approximately 3.5 minutes between each detection for the individual fish. To obtain an even sample rate for the wavelet analysis, depth registrations were averaged by the hour at the individual level. After aggregating to an hourly resolution, we have a total of 46 977 hourly depth registrations, out of which 44 were interpolated to substitute missing values occurring due to signal-collision or during battery changes and other maintenance operations.

#### 3.3 Vertical behaviour

During the first weeks, depth registrations showed that the fish mostly were found at relatively shallow depths during night-time (Figure 3). In contrast, the fish showed a much wider vertical distribution during the day, coinciding with the timing of natural daylight and feeding (Figure 3). This pattern changed abruptly at the onset of artificial underwater lights on 20<sup>th</sup> and 22<sup>nd</sup> December, after which the typical diurnal pattern for the depth registrations disappeared (Figure 3 & 4 and appendix A).

The onset of underwater lights affected depth registrations more at night-time than at day-time. The average depth registrations for all individuals during night decreased by 6.0, 3.5, and 6.8 m for cage A, B, and C, respectively (Table I), from the week before versus the week after the onset of underwater lights. No large difference in day-time depth distribution was observed (Table I).

#### 3.4 Wavelet analysis and phase coherence

The average of the individual wavelet spectra in all three cages shows a distinct period of 24-hours, from the beginning of the experiment until the onset of underwater lights (Figure 5). It is also evident that this periodic signal disappears at the onset of underwater lights on the 20<sup>th</sup> and 22<sup>nd</sup> December 2016 (Figure 5). It should be noted that there is also a 12-hour periodic signal, but this is probably an artefact due to the 24-hour period not being perfectly represented in the wavelet basis by one function, hence signals for the higher-order harmonics are expected (Ryan, 2014).



At the individual level, there was a strong phase coherence for the 24-hour period during the first part of the experiment (Figure 6). This means that the individuals, in general, showed the same, repeated diurnal pattern, staying at shallower depths during night than day. If there had been individual fish who instead resided at shallower depths during day than night, this would have shown up as a difference in phase angle. The coherence ceased after the onset of underwater lights on 20<sup>th</sup> December in cage A (Figure 6) and the 22<sup>nd</sup> December for cage B and C (appendix B).

#### 4. Discussion

This study reveals that the tagged Atlantic salmon exhibited a consistent diurnal behaviour concerning depth usage in the cages, with swimming depths for all individuals following similar diurnal patterns before the onset of artificial underwater lights. Depth registrations in all three cages indicate that the fish typically reside in shallow waters during night. This breaks up in the morning with increasing activity, as indicated by a significantly wider depth distribution throughout the day-time hours, coinciding with feeding. Immediately after the underwater lights were switched on, there was a pronounced change in the depth registrations during night towards deeper recordings and a cessation of the diurnal patterns detected by the wavelet analysis. The recorded swimming depth time-series revealed a sudden disruption of this 24-hour periodic pattern on the 20<sup>th</sup> and 22<sup>nd</sup> December, which correspond to the dates of the onset of artificial underwater lights in the three cages. Environmental data collected during the experimental period indicated that temperature, salinity, and dissolved oxygen were relatively stable and within levels that can be considered normal for the time of year (Golmen *et al.* 2019). The environmental conditions experienced at the farm site were thus unlikely to cause any sudden changes in the behaviour of the Atlantic salmon (Oppedal *et al.* 2007; Oldham *et al.* 2017).

##### 4.1 Response to artificial lights

Natural light conditions along the Norwegian coast vary distinctly with seasons. Moreover, diel behavioural patterns observed in farmed Atlantic salmon have been shown to correlate strongly with light conditions (Huse and Holm, 1993). Several earlier studies reveal that farmed salmon actively seek the deeper parts of the sea cage during day-time, while they ascend on fading light, with a reduction in swimming speed during dusk and night-time (Oppedal *et al.* 2001; Juell and Fosseidengen 2004). The strong effect of underwater light on behaviour found in the current study is consistent with earlier findings on swimming depth patterns, showing strong influences of artificial underwater light, especially during winter months (Oppedal *et al.* 2007; Korsøen *et al.* 2012; Frenzl *et al.* 2014; Stien *et al.* 2014). Using sonars, Juell and Fosseidengen (2004) found that the swimming depth of farmed salmon in two cages with underwater lights at depths of 15 and 3 meters was related to light depth. It was also shown that swimming depth corresponded to changes in the light conditions, where lowering lights from 1 to 15 meters triggered an instant response in depth registrations of Atlantic salmon. The depth response after the onset of light in the current experiment revealed a difference in average depth

over the three cages, where cage B had a shallower depth pattern compared to cage A and C (Figure 4). A possible explanation for this result is that the experiment was conducted in full scale production units, and the lights were set at approximately 8 meters, giving some room for error.

Wavelet analysis was also applied to the extended time-series of swimming depth data lasting until 30<sup>th</sup> March 2017, except for the period between 2<sup>nd</sup> and 15<sup>th</sup> February 2017 when the telemetry system became inoperative due to losses of battery power. When the system came back on, data showed two days with behaviour similar to the depth pattern observed just before 2<sup>nd</sup> February 2017. This was followed by a consistent diurnal depth pattern for all tagged fish in the three cages over nine days, lasting until 27<sup>th</sup> February 2017. After the 27<sup>th</sup> of February 2017, the periodic behaviour suddenly disappeared again for all cages and did not reappear (Appendix C & D). We are not able to explain the sudden change in periodic behaviour in late February based on the farm event log for the three cages. However, problems with the power supply on the facility during this period were reported, but no exact date nor time was noted for this outage. Since the behavioural change occurred in all three cages, a plausible explanation could be an occurrence of an external event such as a power outage and loss of underwater lights. Note also that the increasing intensity and length of natural daylight during this period could have affected the behaviour (Hansen *et al.* 2017). However, such gradually changing factors are not likely to cause abrupt changes in behaviour. Nonetheless, due to the uncertainty in the explanatory factors that could explain this behavioural change, these data were not included in the main analysis of this study.

#### 4.2 Fish behaviour and technology in aquaculture

It is reasonable to expect that healthy farmed Atlantic salmon exhibit normal diurnal depth behaviour through environmentally stable periods. Accordingly, divergence from such behaviour could indicate a change in the farm's rearing environment, e.g. environmental changes, the occurrence of pathogens or other factors affecting fish welfare (Martins *et al.* 2012). Environmental factors known to affect depth preferences in fish are temperature and salinity (Oppedal *et al.* 2007), feeding (Frenzl *et al.* 2014), water flow (Johannesen *et al.* 2020), oxygen saturation (Oldham *et al.* 2017) and light intensity (Rillahan *et al.* 2011; Stien *et al.* 2014). Salmonid alphavirus infections in Atlantic salmon are known to distort normal behavioural traits by causing inappetence and lethargy (McLoughlin and Graham, 2007), which could be manifested as changes in the diurnal day- and night-time depth distributions of the fish. Identification of stressors, both natural and anthropogenic, that result in deviating behaviour, is of key importance in farm management. Tools that enable detection of such incidents at an early stage and provide first signs of warning could potentially have great value in terms of both improved fish welfare and reduced production costs for fish farmers. Here, analysis of movement patterns can be incorporated into the farm management as an indicator of fish welfare by identifying abnormal behaviour at an early stage.

Integrating new technological solutions in the aquaculture industry has been foreseen and encouraged by many studies over the last years (Føre *et al.* 2017; 2018a; 2018b; Hassan *et al.* 2019a; 2019b; Muñoz *et al.* 2020). In acoustic telemetry, miniaturisation of transmitters with multiple sensor options, more robust solutions for signal reception, and extended battery-life for both transmitters and receivers, have provided better opportunities for obtaining longer and more relevant data series on different behavioural traits during entire production cycles. Acoustic transmitters can be fitted with an increasing variety of sensors, providing more elaborate and diverse input data from the fish, e.g. depth, temperature, salinity, acceleration, heart rate, muscle activity (Cooke *et al.* 2016; Hjelmstedt *et al.* 2020; Svendsen *et al.* 2021). The use of tags to monitor fish behaviour have in recent years been debated (Macaulay *et al.* 2021). However, following strict recommendations and protocols during tagging procedures, the research reliability can be strengthened through appropriate measures in surgical protocols in addition to adequate training for the personnel tagging the fish (Hjelmstedt *et al.* 2020; Macaulay *et al.* 2021).

The processing of big data has experienced a boost with the immense increase in computing power and the possibility to store and analyse data in clouds. The precision fish farming (PFF) framework introduced by Føre *et al.* (2018a) describes the implementation of technology in the aquaculture industry based on several underlying core principles inspired by a similar paradigm within the agricultural sector called Precision Livestock Farming (PLF) (Berckmans 2017). Both the PFF and PLF aim to incorporate data collection at the individual level from livestock farming and to provide quantitative and qualitative levels of information for the farmer to aid in decision making (Berckmans 2017; Føre *et al.* 2018a). Hassan *et al.* (2019a; 2019b) launched the Internet of fish-concept (IoF), linking fish behaviour from tagged individuals with a wireless data-transfer that allowed close to real-time fish monitoring over a Low Power Wide Area Network. Combining PFF with wireless and direct transfers (i.e. IoF) with methodology from this study can provide a novel, near real-time way of collecting and analysing data on depth registrations, enabling decision-makers to evaluate and incorporate online data in decisions during production processes.

#### 4.3 Limitations

One limitation of this study is the small sample size, and whether the tagged individuals are a representative sample of the whole population. This is generally the case with acoustic tagging studies. Earlier mentioned studies from other aquaculture facilities such as Føre *et al.* (2018), Leclercq *et al.* (2018) and Muñoz *et al.* (2020) all have relatively low sample sizes (N=31, N=26 and N=10, respectively). Typically, in these types of studies, the sample size is generally low due to the cost of the transmitters and the limited frequency bandwidth. Nevertheless, the observed depth behaviour of the tagged individuals in the current study is in accordance with earlier research (Huse and Holm, 1993), with strong collective diurnal patterns for all tagged individuals. The environmental conditions during the study period were stable and well within the levels that one might expect Atlantic salmon to

exhibit normal behaviour (Oppedal *et al.* 2007; Oldham *et al.* 2017). However, the results may depend on the environmental conditions of the study. For example, factors such as the developmental stage of the fish and water quality may affect fish behaviour. Hence, further studies are necessary to establish whether the results are valid in other environmental settings. Data from two of the 23 tags were lost during the data collection period. One of the tags failed to transmit any signal after deployment and must be considered as a malfunctioning tag. The other tag first registered depth values indicating abnormal behaviour followed by constant depth values, suggesting either sensor failure, tag-repulsion, or mortality, thus resulting in a total tag loss of 4.35 % during the study period. By comparison, the total registered mortality at the farm over the same period was 1.04 %, ranging between 0.34 – 2.13 % in the cages with tagged fish.

#### 4.4 Concluding remarks

The current study demonstrates the possibility to implement pattern recognition in the form of wavelet analysis on depth registrations to both detect and describe divergence from normal diurnal depth behaviour of farmed Atlantic salmon, exemplified by using underwater lights. As farmed salmon typically show strong diurnal behavioural patterns, results from the study show the feasibility to incorporate time-series of depth data as an indicator for normal fish behaviour. Atlantic salmon is the most important aquaculture species in Norway and a comprehensive and more precise understanding of animal behaviour during the production cycle are crucial to accommodate future growth of the industry. Implementation of good monitoring systems is essential in all animal husbandry, and there is an unresolved potential to enhance both fish welfare and production during the ongrowing phase for farmers in aquaculture.

#### **Author contributions:**

**John Birger Ulvund:** Methodology , Formal analysis, Investigation, Writing - Original Draft, Visualization, Writing - Review & Editing

**Solveig Engebretsen:** Methodology, Formal analysis, Writing - Review & Editing, Visualization

**Jo A. Alfredsen:** Methodology, Conceptualization, Writing - Review & Editing

**Torstein Kristensen:** Conceptualization, Writing - Review & Editing, Supervision, Project administration

**Henning A. Urke:** Conceptualization, Supervision, Project administration, Funding acquisition

**Peder A. Jansen:** Formal analysis, Writing - Review & Editing, Visualization

#### **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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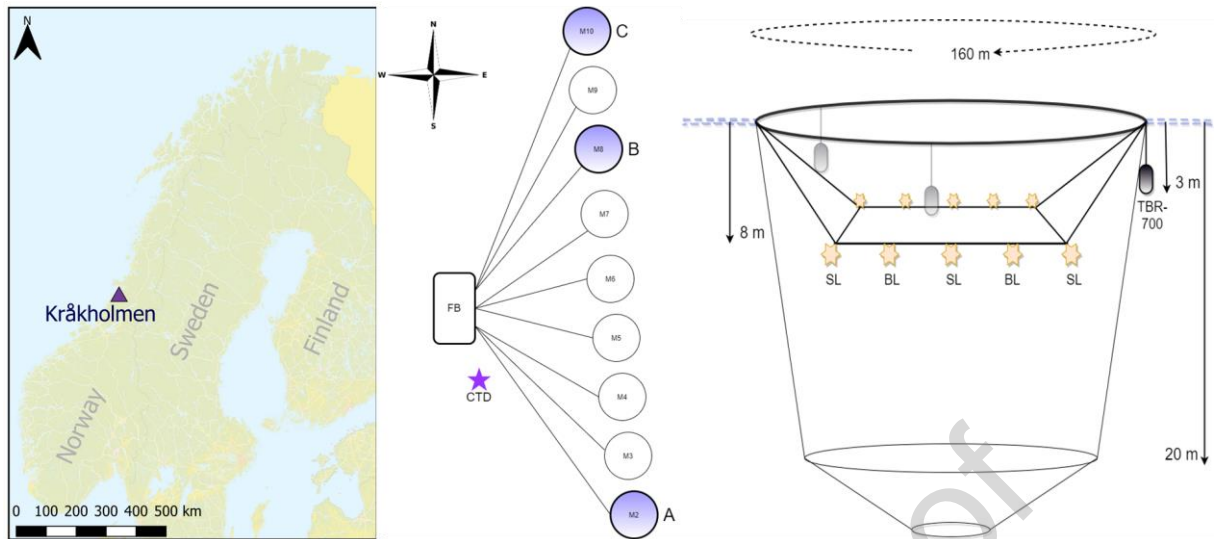
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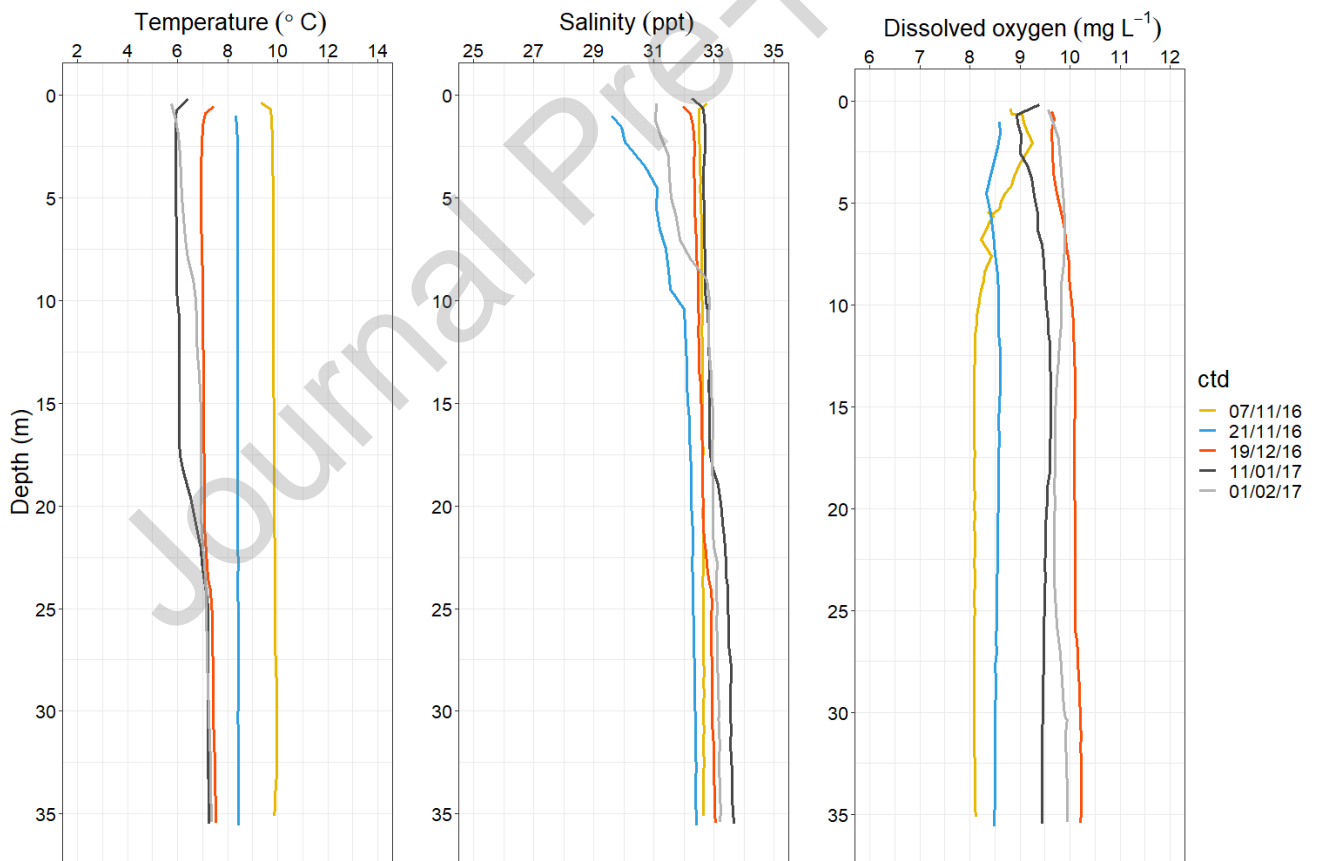
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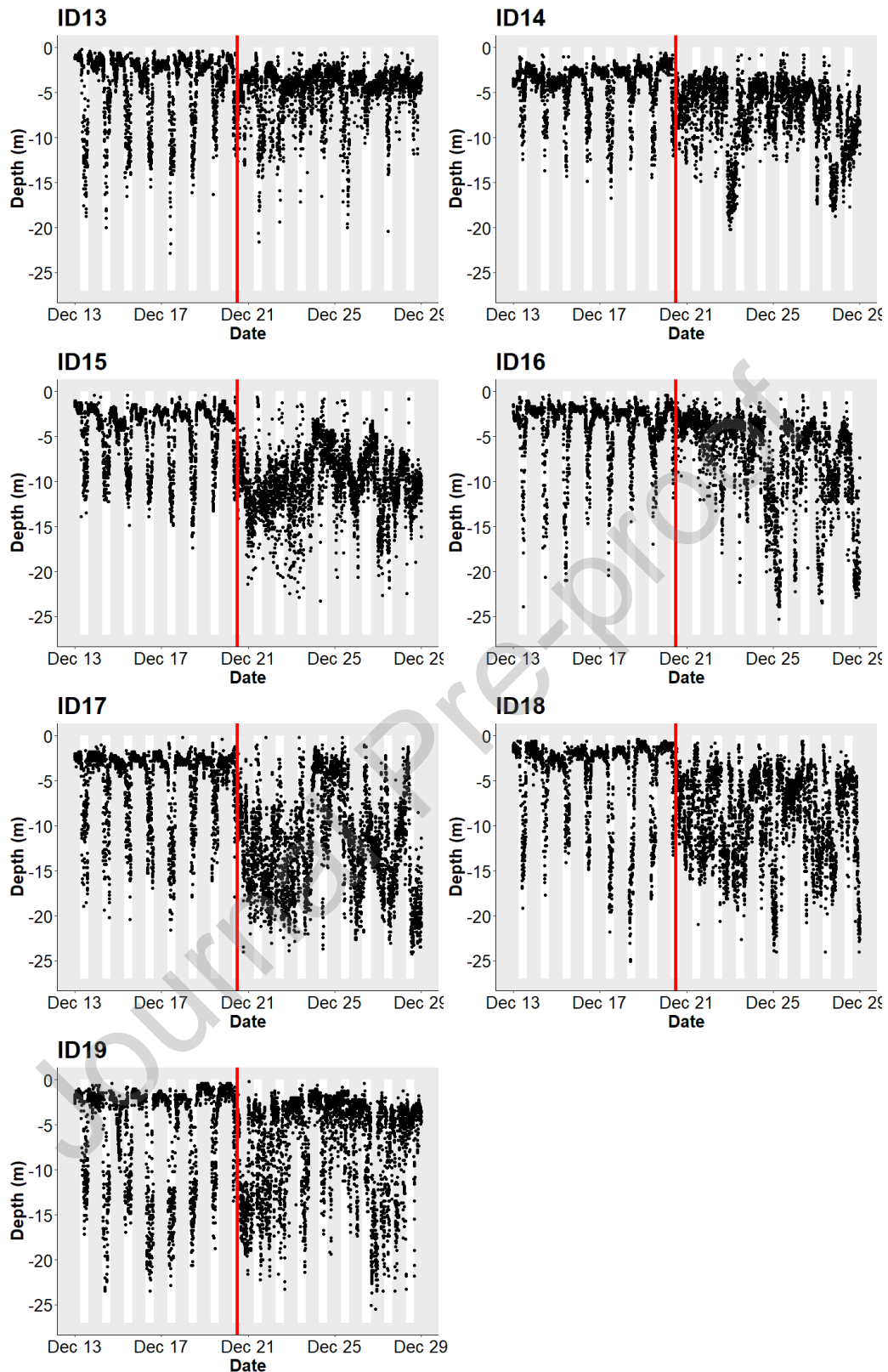




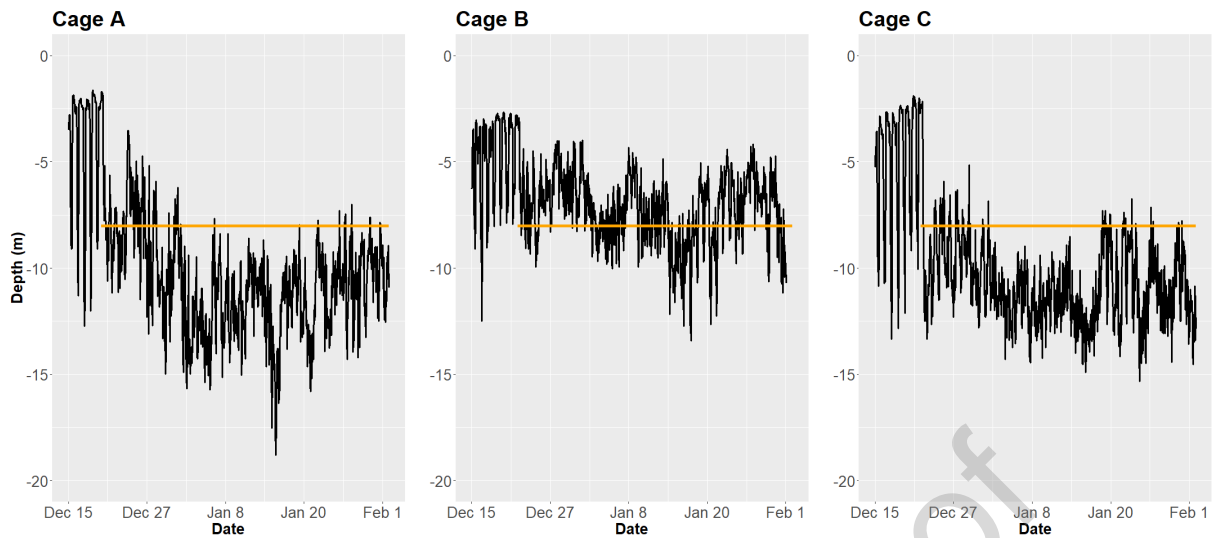
**Figure 1.** Location of the salmon farm Kråkholmen in central Norway (left panel), placement of the experimental cages A, B and C (middle panel) and the setup of underwater lights (right panel). CTDO-profiles were collected at a fixed point indicated by the blue star near the feeding barge (FB, middle panel). Ten lamps were submerged to eight-meter depth in two parallel rows approximately 12 meters apart, spanning the cage from east to west (SL=SubLite, BL=BlueLED, TBR700=Receiver).



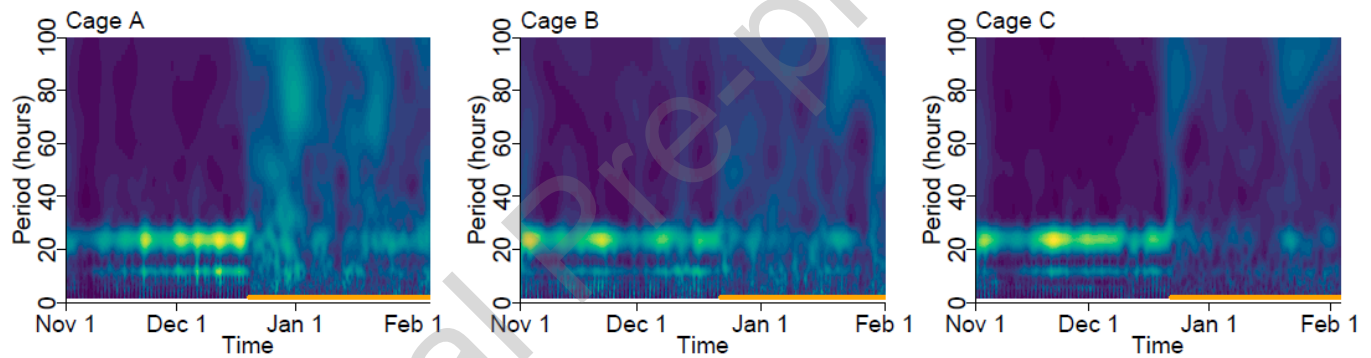
**Figure 2.** Vertical profiles of temperature, salinity, and dissolved oxygen recorded at the experimental site between 2<sup>nd</sup> November - 1<sup>st</sup> February.



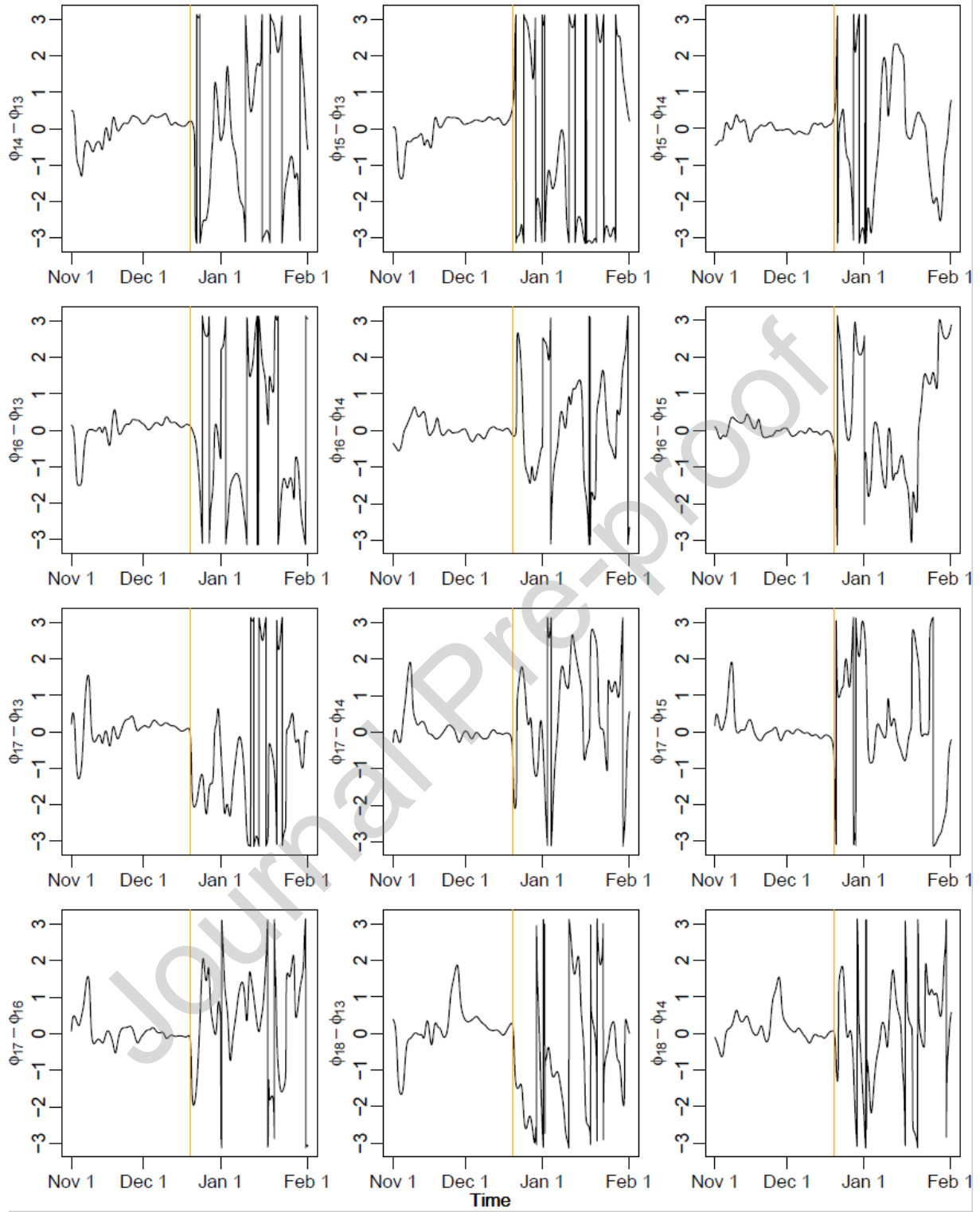
**Figure 3.** Individual depth registrations for all tagged fish in cage A from 13<sup>th</sup> to 29<sup>th</sup> December 2016. Each point represents a depth recording. White vertical boxes indicate daylight, grey vertical boxes indicate night and the red line indicates the onset of underwater lights.

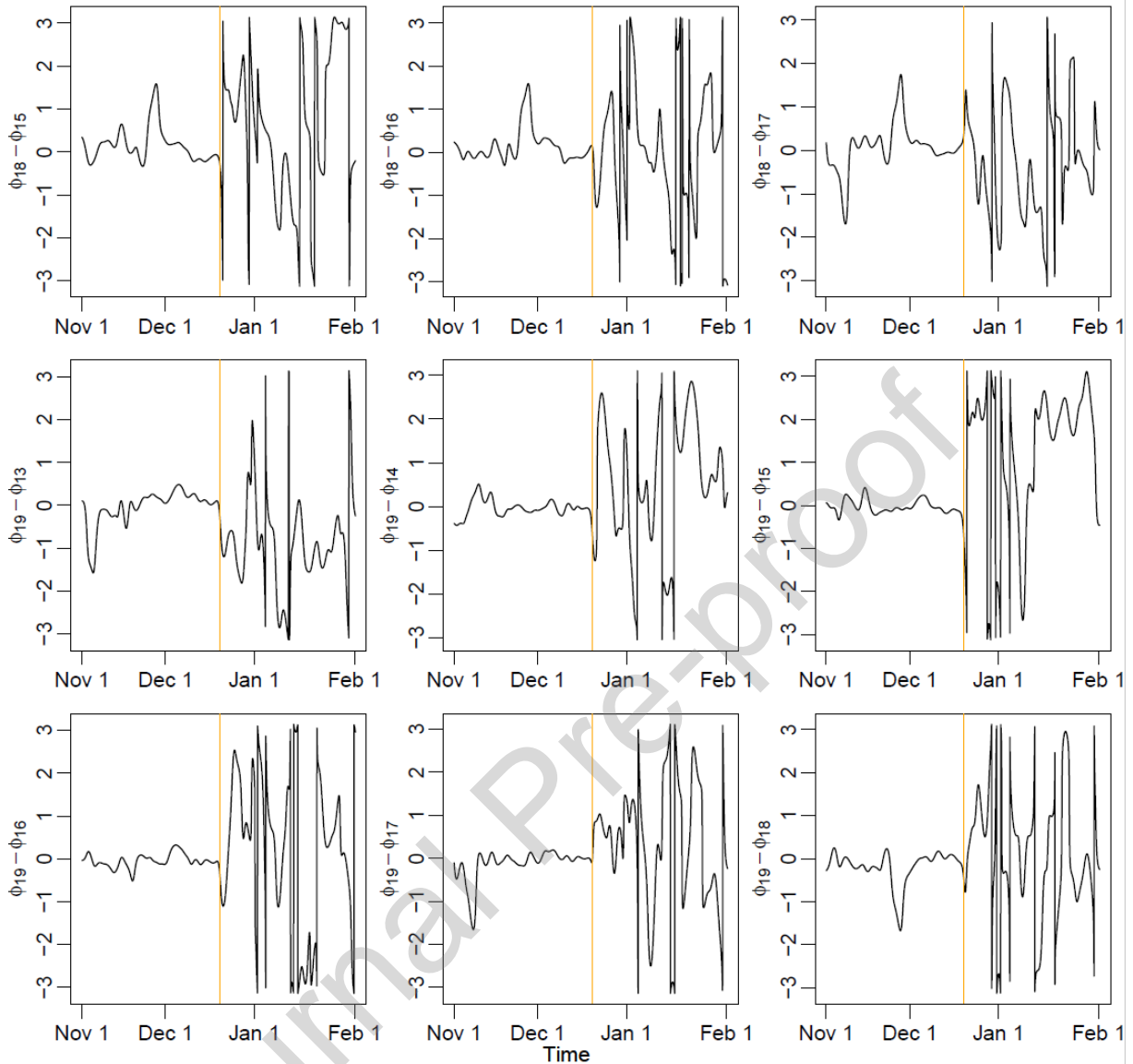


**Figure 4.** Average swimming depth for all tagged fish in the three cages from 15<sup>th</sup> December - 2<sup>nd</sup> February. The orange horizontal lines illustrate the depth and presence of artificial underwater lights.



**Figure 5.** Wavelet spectra for all tagged fish in cages A, B and C, respectively. The orange horizontal lines represent the onset of underwater lights. Lights were activated on the 20<sup>th</sup> December for cage A, and on the 22<sup>nd</sup> of December for cages B and C.





**Figure 6.** Pairwise differences in phase angles for tagged fish in cage A for the 24-hour period.  $\phi_i$  is the phase angle for individual  $i$ . Vertical lines indicate the onset of underwater lights. Phase differences of zero imply synchronous depth use throughout the 24-h period, whereas increasing deviations from zero illustrate decreasing/non-existent coherence between the individuals.

**Table I.** Average swimming depth (m) over all individuals in cage A, B and C one week before and one week after the onset of underwater lights during day and night.

<b>Cage</b>	<b>Day/Night</b>	<b>Week before (depth ±SD)</b>	<b>Week after (depth ±SD)</b>	<b>Change in avg depth</b>
<b>A</b> (N=7)	Day	7.1 ± 4.6	7.6 ± 4.4	0.5
	Night	2.3 ± 0.9	8.3 ± 5.1	6.0
<b>B</b> (N=8)	Day	6.8 ± 4.5	6.1 ± 3.2	-0.7
	Night	3.3 ± 2.2	6.8 ± 4.0	3.5
<b>C</b> (N=6)	Day	8.1 ± 4.7	8.6 ± 4.2	0.5
	Night	2.9 ± 1.4	9.8 ± 3.9	6.8

## Highlights

- Atlantic salmon display strong diurnal depth profiles over the winter months
- Underwater light regimes reveal instant changes in diurnal cycles
- Wavelet analysis detected abrupt changes in behaviour from normal depth behaviour
- Wavelet analysis paired with acoustic telemetry tags as a surveillance tool for fish behaviour

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