Deliverable 6.4 (M54)

Title

General determination of welfare indicators across the fish species using the monitoring technologies in tanks and cages

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Contents

1. Objective

The deliverable aims to experimentally explore the possibilities of monitoring technologies to detect the fish stress event among different species (salmon, sea bass, seabream) and different production units (tanks and cages). The deliverable concludes on the possible use of monitoring technologies in tanks and cages to determine welfare indicators across fish species, which can be used as a guide for researchers to improve welfare indicator monitoring.

2. Background

Fish welfare is a well-recognized concept that has gained significant attention in recent years, especially within research and the aquaculture industry. In the context of evaluating monitoring technologies — the main focus of this deliverable — we adopt a welfare definition centred around the animal's emotional (or emotion-like) states. According to this perspective, good welfare involves minimizing negative experiences (like stress or fear) while promoting positive ones (such as social interactions in species that thrive in groups). Ensuring good fish welfare is fundamental to achieving sustainable aquaculture and reliable research results. The AQUAEXCEL3.0 project aims to enhance aquaculture infrastructure and standardize practices through collaborative research efforts. Specifically, Work Package 6 (WP6) focuses on harmonizing knowledge about welfare indicators across the most important European aquaculture species and improving fish welfare in experiments.

Several activities were done in WP6 to reach this goal. Deliverable D6.1 summarised the existing and emerging technologies available for welfare indicators monitoring. In D6.2, we defined the guidelines for important operational welfare indicators for key European species used in aquaculture research. To demonstrate how modern technologies like video monitoring, echo sounders or data logging tags can detect changes in operational welfare indicators during short-term fish stress, several harmonized experiments were planned. The experimental settings and plans were described in milestone M33. This deliverable describes the data recorded during the experiment, the data processing methods and the obtained results.

3. Methodology

The experiments and data collection described in the next paragraph were conducted at four institutes: HCMR, NOFIMA, SINTEF, and CSIC. Table 1 summarizes the experiments.

Table 1. Summary of the experimental sites, sensors and fish species.

		HCMR	CSIC	NOFIMA	SINTEF
Sea cage	**Video**	Seabass	x	x	Salmon
	Echo sounder		x	x	
Tank	**Video**	Seabass	Sea bream	Salmon	x
	Tag	x			x

The experiments in sea cages were done in Norway (SINTEF) with Atlantic salmon (*Salmo salar*) and in Greece (HCMR) with European seabass (*Dicentrarchus labrax*). Video monitoring was used at both sites, but the echosounder was only by SINTEF. The fish crowding and net cleaning were used as short-time fish stressors at both sites.
The experiments in tanks were done in Norway (NOFIMA) with Atlantic salmon, Greece (HCMR) with European seabass and Spain (CSIC) with sea bream (*Sparus aurata*). The video monitoring was used at all sites, but the tags were used only by NOFIMA and CSIC. The fish crowding was used as a short-time fish stressor at all sites.
The aim was to compare the same technologies for different cultivation units and different species but with similar stressors. Therefore, fish crowding was used as a stressor in all experiments. Still, due to site and species specificities, not all sites were able to use the same monitoring technologies and follow the same experimental plan.

3.1. Data collection

3.1.1. Cages

SINTEF (Norway)

The experiment took place at Tristeinen (Frohavet, mid-Norway), one of the SINTEF ACE research farm sites. Fish behaviour measurements were conducted between *4.4.2023* and *19.05.2023* in pen 8, located in the middle of 10 net pens installed in a double-row perpendicular to the main current direction. The pen had a circumference of 160 m with straight side walls of 15 m depth stabilised by a bottom ring. The net cone below had a depth of 15 m, giving the pen a total depth of 30 m and a volume of 40 856 m^3. The pen contained approx. 190 000 Atlantic salmon (*Salmo salar*) with an

average weight of 1.7 kg resulting in a stocking density of 7,9 kg m^{-3}. The lice skirt installed around the pen was lifted between 7th of April and 3rd of May to allow good water transport through the pen and ensure sufficient oxygenation during the experimental period and specifically during the crowding phases. Fish were typically fed for 3 hrs using a surface feed spreader, remotely controlled from a land-based feed centre. As feeding has a strong impact on fish behaviour (e.g., location in the pen), specific feeding times were recorded and included in the analyses of fish behaviour.

After a 13-day baseline period where the typical behaviour of the fish was recorded, net cleaning and inspection were conducted (Fig. 1). On the next day, the bottom ring of the net was raised, effectively shortening the upper net walls from 15 m to 7 m, reducing the pen volume to 60% and increasing fish density 1.7 times to 13.2 kg m^{-3}. After 24 hrs, the upper net walls were shortened further to 3 m, reducing the pen volume to 40% and resulting in 2.5 x the fish density at 19.8 kg m^{-3}. After 24 hrs, the net was returned to its original shape and inspected. The operation of raising/lowering the net took approx. 60-90 mins and was typically started at 8 am on the day. After a 7-day recovery period, the net was again cleaned and inspected. Three days later, the net was raised for two 24-hour periods, similarly to before, then returned to its original state and inspected. After an 8-day recovery period, the net was cleaned. The monitoring stopped nine days after that.
Crowding was conducted by experienced farm personnel, and fish behaviour and oxygen concentrations in the pen were monitored throughout the entire duration so that the operation could have been cancelled at signs of impacted welfare. At no point in time did the volume reduction exceed the allowed maximum stocking density of 25 kg m^{-3}.
Net cleaning was conducted using an in-situ pressure washing rig (FNC, AkvaGroup) and took approx. 2 hrs. The cleaner is equipped with cameras and is used simultaneously for inspection of the net. Inspection after net cleaning was conducted without active use of the washing units and took approx. 90 mins.

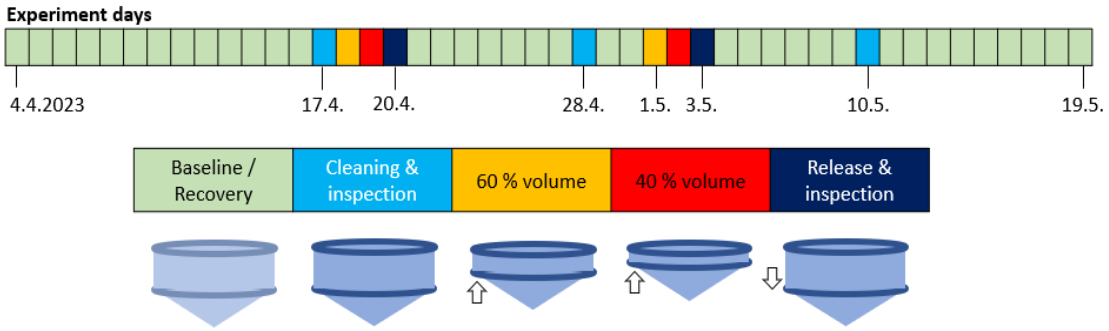


Figure 1: *Experimental timeline. Experimental duration of 46 days (4.4. to 19.5.2023) indicating timing of net cleaning, inspection, and crowding days (with volume reduction to 60% and 40%).*

Fish behaviour was monitored using an underwater camera and four echosounders installed in the pen (Fig. 2).
The **camera** (Fyssalis V5, Elementtech, Greece) was mounted on a gimbal and installed at approx. 5 m depth. 10 m from the net wall, looking up towards the surface. The camera was operated using Intel Nuc running SmartPSS software for video acquisition (at 25 FPS at 1080x1920) between 6 am and 6 pm. In addition to saving data on a local hard drive, an online live feed was available.

Wind data (direction, middle wind and wind gust) from Halten Fyr was downloaded from seklima.met.no after the confinement trials ended. The data has a spatial resolution of one hour and a measurement period from trial start to end.

Four **echosounders** of the type EK15 200-28CM (SIMRAD) were installed, pointing downwards from the surface with even spacing on a diagonal mooring rope that crossed 1/3 of the pen surface.

To monitor environmental conditions and ensure fish safety, oxygen was monitored in three places: (i) in the centre of the echosounder mooring rope, a string with four oxygen sensors (Innovasea, Norway) at 1m, 5m, 8m, and 15m was installed. (ii) An additional string of two oxygen sensors was installed 5m from the cage wall with sensors at 1m and 5m depth. (iii) Outside the net pen in the middle of the farm array, a reference station with four oxygen sensors at 1m, 5m, 8m, and 15m was installed. Oxygen values never dropped below normal values and are not presented here.

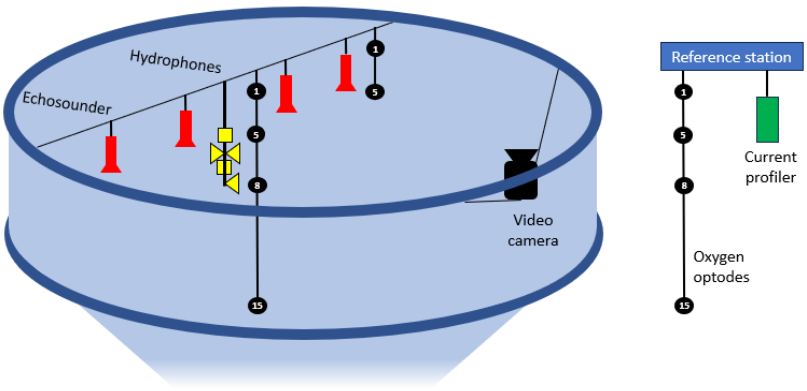


***Figure 2**: Monitoring set-up in SINTEF. Overview of the sensors installed in the net pen and at the reference station (not to scale).*

HCMR (Greece)

Figure 3. *Experimental site and camera setup at HCMR.*

A group of approximately 22,000 European seabass (*Dicentrarchus labrax*, approximately 350 g body weight) was reared at a stocking density of 7.5 kg m^{-3} in a circular polyester cage with a diameter of 40 m and a depth of 9 m. The cage featured a cylindrical net extending to a depth of 8 m, with a closing cone at the bottom measuring 1 m. The cage (Figure 3) was located at the pilot-scale farm of HCMR, an aquaculture facility certified by the national veterinary authority (code: GR94FISH0001), situated in Souda Bay, Crete (35.4800464N, 24.1117347E). The E. seabass stock originated from the Mesocosm hatchery of HCMR. After larval rearing and pre-growing, juveniles with an average weight of approximately 2 g were transferred to the pilot-scale farm at 120 days post-hatching.

A submerged network camera (Fyssalis v3.1; Figure 3) capturing at 25 fps was used for monitoring and continuous video recording during daylight hours (06:00 – 18:00). The image resolution was 1280 x 720 pixels. The camera was positioned at 4m depth using a gyroscopic gimbal stabilizer to ensure upward positioning. The recording was continuous during the daylight hours. The videos were collected using RTSP streaming and were analysed locally at the institute's facilities.

The experimental procedure was conducted in November 2022 and involved three sequential stress treatments over three consecutive days. On the first day, stress was induced by cleaning the cage net. A generator was started, and once stabilized, it powered a pump connected to a drum (a disc with water outlets), which sprayed pressurized water onto the net. The entire cleaning process lasted approximately 45 minutes.

On the second day, stress was induced by reducing the cage volume by 25% (2 m up), and on the third day, the volume was further reduced by 50%, i.e., reaching 15 kg/m^3 (Figure 4). The volume reduction was achieved by raising the bottom of the cage to the designated level. The net was returned to its original state the following day after the 50% reduction. The experiment was repeated after two weeks to assess any differences in reactions. Throughout the procedure, a diver inspected the net for

potential damage caused by crowding. Feeding was taking place three times daily (at 08:00, at 12:00 and at 16:00).

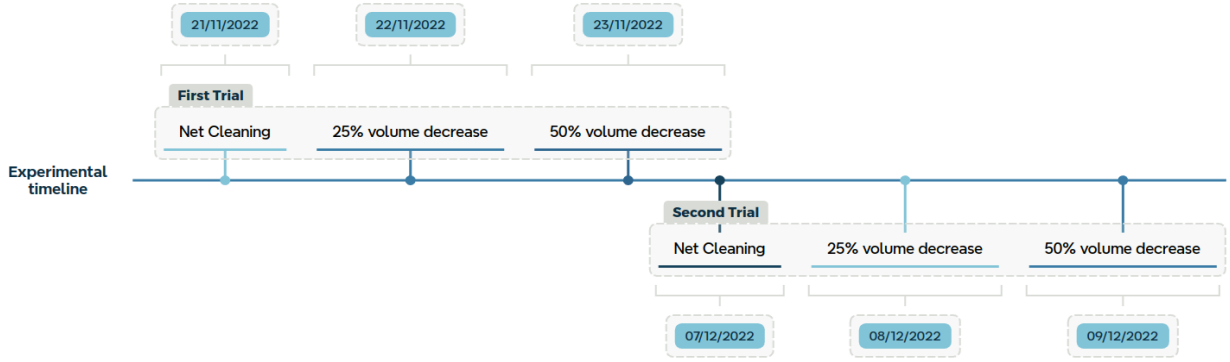


Figure 4. *Experimental timeline for the seabass stress experiment in cages at HCMR.*

Simultaneously with fish monitoring, continuous environmental monitoring (of dissolved oxygen and temperature) was conducted. The purpose of this monitoring was to identify any behavioural changes unrelated to the stressors.

3.1.2. Tanks

HCMR (Greece)

A trial was performed in the marine Recirculating Aquaculture System (RAS) of HCMR (Greece), where juvenile European seabass of approximately 250 g were subjected to a protocol of repeated stressing, induced via crowding, at intervals of 15 days over a two-month period. Over this period, growth performance was monitored (weight measurements, FCR) along with visual inspection for external abnormalities (ulcers, scales, fins, and tail issues). At sampling points, blood from both groups (the control and the stressed group) was collected from the caudal vein centrifuged, and plasma cortisol measurements were performed using commercial kits to quantify the baseline stress levels. For the duration of the trial, the behaviour of the fish was recorded via IP cameras, and behavioural indicators such as speed were analysed using a methodology previously developed (Georgopoulou et al., 2024). The fish were hand fed to apparent satiation, and the feeding schedule was twice per day, at 08.30 - 09:30 and 14:00-15:00.

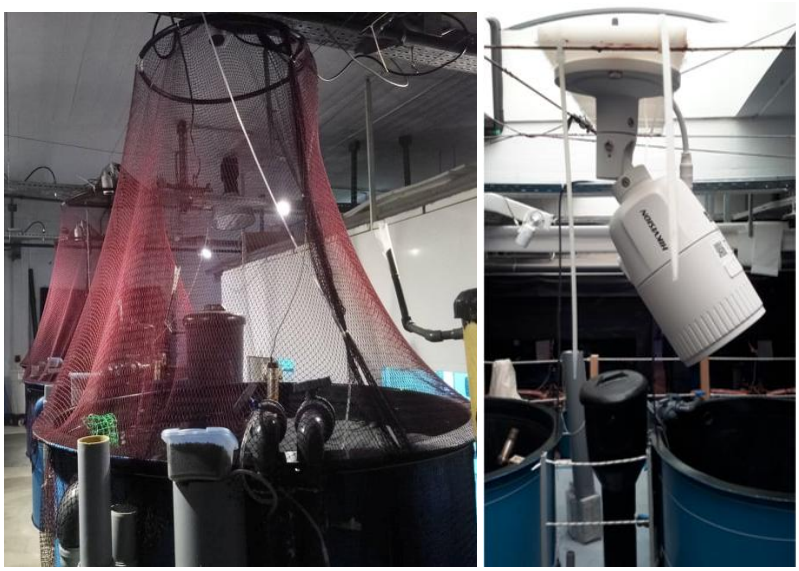


Figure 5. *The experimental setup of the tanks at HCMR (left) and the camera (right).*

In more detail, 240 fish (of 267±30 g weight and 33±3 cm length) arrived from the HCMR pilot scale farm on the 3rd of October 2022 and were kept at the AquaLab facility of the HCMR, a certified laboratory for fish rearing (EL91-BIOexp-04) in accordance with legal regulations (EU Directive 2010/63) and after approval by the Ethics Committee of the IMBBC and the relevant veterinary authorities (Ref Number 255344). The fish were divided into 6 groups of 40 fish and were randomly distributed into 6 cylindroconical tanks of 2 m^3 volume and 1.5 m diameter at a thermoregulated marine RAS (see Figure 5) and kept under typical rearing conditions for the species (T = 20°C, pH = 8.0, salinity = 37 psu, and a 12 h L:12 h D photoperiod cycle). Three of the tanks were used as the control and three as the treated group. The groups were monitored using network cameras (HIKVISION DS-2CD1623G0-IZS) capturing at 6 fps with frame resolution 1280x720 for a period of one month (from 27 October to 27 November 2022), from 08:00 to 19:30. The cameras were positioned over the tanks, pointing downwards (Figure 5). Due to network connectivity issues in the IP cameras, we ended up using 4 out of the 6 tanks (two control and two treated).

During normal days, fish were fed twice a day (~08:40 and ~14:40). In addition, there was human presence in the facility between 08:00 and 15:00 every day except Sundays. Fish were left to acclimate for a month before the stress trial occurred.

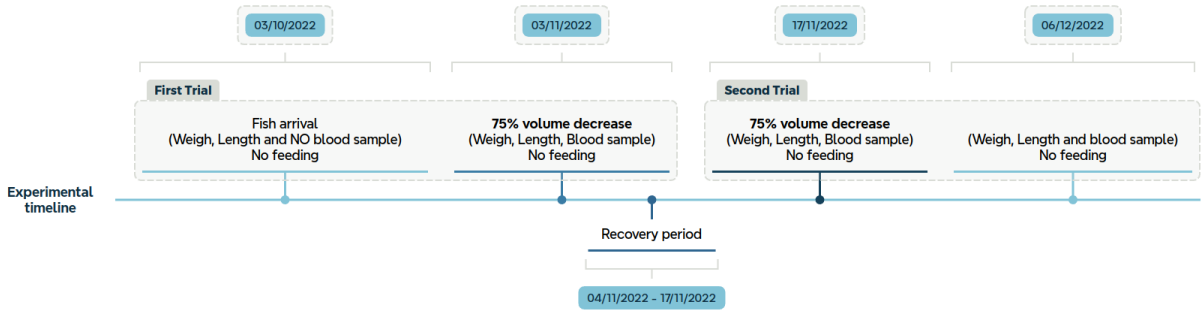


***Figure 6**. Experimental timeline for the seabass experiment in RAS at HCMR.*

During the stress, the tank volume was reduced by 75% (reaching a stocking density of 50kg/ m^3) using a triangular net cage, as shown in Figure 7. The fish remained in the cage for a period of 30 minutes, and they were released immediately after this. In each trial day and before the stress event, 8 fish were randomly sampled from each tank (including the control groups) for weight and cortisol determination (Figure 6).

Figure 7. *Stress induction in RAS at HCMR.*

CSIC (Spain)

Three trials were conducted at the Institute of Aquaculture Torre de la Sal (IATS-CSIC) infrastructure in the summer seasons of 2021 and 2022, with fish maintained in a 3,000L tanks flow-through system with natural photoperiod and water temperature conditions at IATS-CSIC (40° 5'N; 0° 10'E). The first trial (summer 2021) explored the effects of high stocking density and concurrent low oxygen availability on the welfare, physiological responses, and behavioural adaptations of gilthead sea bream, considering low (LD, 8.5 kg/m^3), medium (MD, 17 kg/m^3), and high stocking density (HD, 25 kg/m^3). Oxygen levels ranged from 3 to 6 ppm, depending on stocking density. The two trials conducted in the summer of 2022 focused on species-specific (gilthead sea bream and European sea bass) stress responsiveness and the habituation to a repetitive stressor. The main outcomes of these works have been published in two Open Access publications: Holhorea et al., 2023, Frontiers in Physiology 14:1272267, doi: 10.3389/fphys.2023.1272267, and Holhorea et al., 2024, Biology 13:879, 2024, doi: 10.3390/biology13110879.

Effects of high stocking densities and limited oxygen availability on gilthead sea bream were assessed by means of biometric, behavioural, physiological, and tissue damage assessments. Behavioural monitoring was conducted using the tri-axial accelerometer-based data logger AEFishBIT attached to the operculum of 12 randomly selected fish per experimental condition. Devices were programmed for data acquisition of physical activity and respiratory frequency for 2 min every 15 min for two consecutive days, in which fish remained unfed. The sampling frequency of the AEFishBIT device was 100 Hz. At the end of the recording period, all AEFishBIT devices were successfully recovered, and pre-processed data was downloaded to track the recorded behavioural traits. Growth performance was assessed through weight measurements, specific growth rate (SGR), and feed conversion ratio (FCR). Blood samples were analyzed for glucose and cortisol levels, and liver and muscle tissue samples were collected for gene expression profiling. Additionally, fish were photographed and scored for external tissue damage, fin erosion, and skin lesions.

In the trials conducted in the summer of 2022, high-quality 4K video cameras (Vivotek FD9391 EHTV, at 2560 x 1440 resolution) were placed on top of each tank, covering their entire volume. Camera placement was optimized to avoid light reflection that could distort the recording. Distortion due to air bubbling was also minimized. The species-specific stress responsiveness was studied in three-year-old gilthead sea bream (mean weight 1200 g) and European sea bass (mean weight 950 g) in similar conditions of temperature, O_2 concentration (4.5-5 ppm), and stocking density (18-20 kg/m^3) cultivated in 3000l tank. Behavioural responses were assessed before, during, and after exposure to a confinement stressor. The confinement stress test was designed to be applied as a reproducible and single/repetitive stressor without directly handling the fish or compromising water quality. This test consisted of a temporary (45-minute) 66% reduction of available space by means of a self-made PVC structure during two consecutive days (illustrative video available at https://vimeo.com/1015372239); see Fig 8.

Figure 8. *Left- Fish cultivation tank with sea beam at CSIC. Right - confinement to 33% of the volume.*

The structure was removed after confinement, enabling the fish to resume free swimming. The assessment of the behavioural response was performed using the AEFishBIT data logger, externally attached to the operculum of 12 fish of each species one day before the stress test, for the simultaneous monitoring of physical activity and respiratory frequency every 15 minutes over two days. Camera recorded during one hour before confinement, the confinement procedure and three additional hours after the end of confinement. Growth and physiological parameters were also measured, including body weight, feed intake, muscle fat content, and external tissue damage. Hematological analyses included hematocrit, hemoglobin, and gene expression profiling of stress-related markers in liver and muscle samples.

The habituation to high stocking density was assessed using gilthead sea bream individuals reared at different stocking densities (CTRL: 10 kg/m^3; high density, HD: 18 kg/m^3) for over two months prior to being subjected to the repetitive stressor stimulus on two consecutive days. For 8 fish per experimental condition, blood samples were taken to assess the haematocrit (Ht) and haemoglobin (Hb) concentrations. Muscle fat content was determined, and photographs were taken to evaluate the external damage of fish (cataracts, exophthalmia, gill status, fin damage, and skin lesions; n=28 fish per condition). Portions of dorsal white skeletal muscle were collected for gene expression analyses. Four days after sampling, AEFishBIT devices (20 per experimental condition) were externally attached to the operculum of anaesthetized fish (2 min every 15 min for 2 days. Sampling frequency 100 Hz), and fish from both stocking densities were exposed to the repetitive stressor during two consecutive days. Data analysis was conducted considering three time periods during the light phase of the day: pre-stress (3 hours), stress (60 minutes from structure placement until structure removal), and post-stress (9 h).

NOFIMA (Norway)

NOFIMA – Fish were crowded three times over a 75-day period, 14 days between each crowding. For crowding, frames were used to decrease the tank volume by 25% for 30 minutes. The setup and the fish confinement are shown in Fig. 9

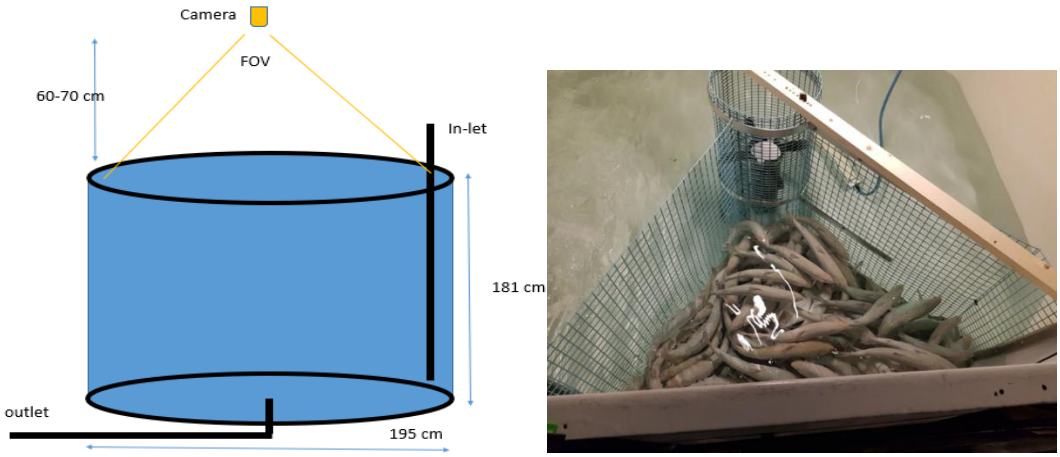


Figure9 *Experiment setup and the stressing samples at Nofima*

Behaviour monitoring: Two technologies were used for welfare change monitoring in the tanks. The video cameras and the data logger. The video camera was placed above the tank and pointed down to the water surface. It recorded the changes in fish behaviour continuously during the whole experimental period. Only the day period was recorded. The records were divided into short video footage (~15 minutes) to simplify data processing. It was not able to record during the fish crowding operation, but the record was made immediately after the operation finished. The data logger was used to monitor fish swimming activity (acceleration in two axes and fish breathing activity). The records were synchronized with camera records using the date-time stamps.

- Video camera: - GoPro camera was placed 60-70 cm above the tank to cover the whole tank space. The data was recorded from video separated for each tank. The records were marked by date/time stamp to synchronize them with the stressor event and fish manipulation.

Simultaneously with the fish monitoring, environmental conditions were monitored continuously. The purpose of environmental monitoring was to detect behavioural changes not related to the stressors. Fish crowding, feeding, and fish manipulation could influence fish behaviour, and temperature, oxygen, and pH levels were taken care of.

3.2. Data processing

3.3. Video

To compare the results of video data analysis for different species and from different sites, we defined the basic parameters to extract from video footage. Different techniques were used for different datasets. Classical image processing techniques based on background subtraction and object detection were compared with modern approaches based on convolutional neural networks.

Both the group behaviour and individual fish behaviour parameters were used. The following are parameters that describe the general trend of behaviour considering the collective action of fishes in the group. The parameters for individual fish are estimated over short tracks and distances, then aggregated for group parameterization, with the average value serving as the main metric.

Parameters:

1. *Cohesion*: It will provide the space occupied by fishes, which helps to determine the closeness of the grouping of fishes within their respective environments. This is important to understand how they interact with one another and space, which influences their welfare.

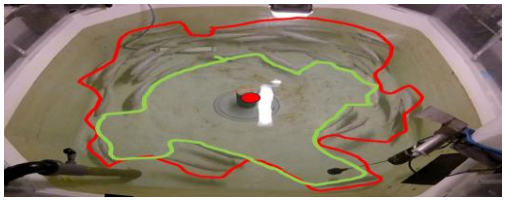


Fig 10: Sample cohesion from the data

2. *Position*: The fish's location within the experimental environment (tank or cage) provides insight into their movement patterns and spatial distribution. The position is determined using the (x, y) centroid of each detected object (fish). The centroid is extracted using any tool that finds the detected blob's centre (object).

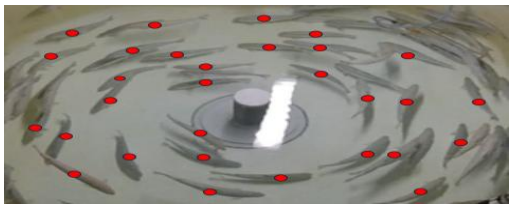


Fig 11: Sample positions of the fish.

3. *Speed* (Activity): Speed is calculated using the displacement of the object's centroid between consecutive frames. Speed is an important indicator of activity levels and can be correlated with fish welfare, as reduced movement may indicate stress or poor health.

Fig 12: Sample fish movement.

4. *Direction* (Orientation): This parameter evaluates the fish's movement relative to the expected or intended direction. A significant deviation from expected orientation can signal distress or difficulty in navigating the environment, which may be a sign of compromised welfare. This can be achieved by estimating orientation using the major axis of the detected object**.**
5. *Distance from the Centroid*: This measures how far each fish is from the central point of the tank or cage. It is a useful parameter for detecting isolation or separation from the group, which could indicate social stress or individual welfare issues.

Fig 13: Sample fish orientation.

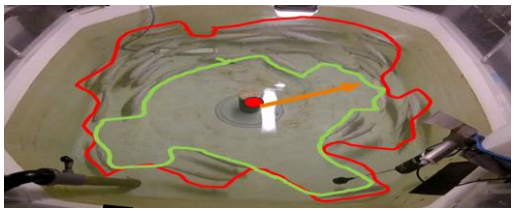


Fig 14: Sample fish distance from the tank centre.

By monitoring these variables, we aim to create a comprehensive profile of fish welfare under varying conditions in both tanks and cages. To achieve the project's objectives, these data processing as welfare indicators are proposed for computation across various fish species using monitoring technologies in tanks and cages, as discussed in the following sub-sections.

NOFIMA (Norway) - Tank

A YOLOv8-based pose estimation model was trained to estimate key anatomical landmarks—snout, dorsal fin, and tailbase—in fish. The trained model was then applied to video frames sampled at 1-second intervals to detect these key points. Although extensive experimental data were available, many datasets lacked recordings from the post-crowding period, limiting their suitability for further analysis. Consequently, only five crowding events (as detailed in **Table 2**) were selected for analysis.

Table 2. *Details on crowding events*.

Dates	**Tank-108**	**Tank-113**	**Tank-115**
24.05.2023	No significant post-crowding data	No significant post-crowding data	Post-crowding data available
08.06.2023	No significant post-crowding data	Post-crowding data available	No significant post-crowding data

23.06.2023	Post-crowding data available	Post-crowding data available	Post-crowding data available

Through the detections, initial plans included utilizing features such as the centre of mass of the area occupied by the fish, cohesion, direction, and speed. However, preliminary analysis indicated that direction, which represents the swimming orientation, provided the most significant insights into crowding effects. Consequently, further analysis focused on directional changes and how they were influenced during crowding events.

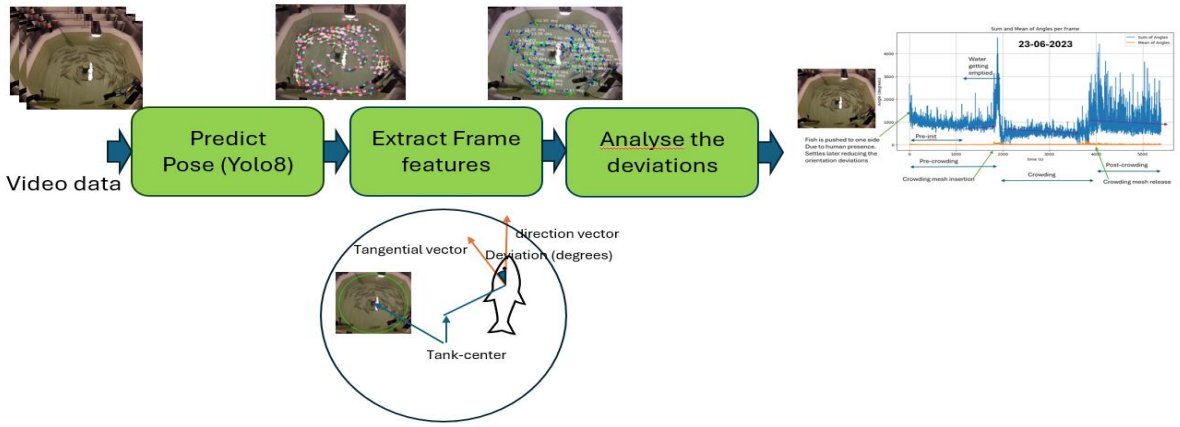


Figure 15. *The data processing pipeline is shown here. The frame features are the mean and sum of the deviations of detection per frame and have been used to analyse the fish behaviour during the entire crowding experiments.*

The pipeline of the process has been depicted in Fig. 15. The centre of the tanks was marked using the developed tool, and a direction vector was computed for each fish based on the detection, defined as the vector from the dorsal fin to the snout. The deviation was measured as the difference between this direction vector and the tangential vector relative to the tank centre, as illustrated in the corresponding figure.

For the selected crowding events, detections were obtained for all frames sampled at 1-second intervals. In each frame, the deviation from the tangential vector was computed for every detected fish based on the dorsal fin orientation. To quantify these deviations, two features were extracted per frame: the mean deviation and the sum of all deviations across detected fish. These features were then plotted against time to track variations throughout the crowding events.

This analysis aims to identify changes in these features, as deviations from expected swimming behaviour may indicate anomalies in the dataset associated with crowding effects.

We processed the video data using the statistical background subtraction method to compare the CNN-based and classical image processing approaches. The following STEPS (1) is used to compute the

recorded data in order to extract relevant information. The analysis follows a traditional approach using MATLAB as the coding platform. Our methodology was performed, see Fig 15. It includes background subtraction to separate moving objects from a static background and motion-based segmentation using MATLAB's vision. Foreground detector is used for object detection, and morphological processing is used to refine segmentation by removing noise and smoothing object edges. Finally, we employ Blob Analysis for tracking small objects, ensuring accurate measurement of parameters such as position, orientation, area, distance, and speed.

STEPS (1) Analysis for Fish Behavior Analysis

1. **Process Video Frames:** *Read and resize frames - resized to **25%** of their original size using imresize(frame, 0.25).*

   ***à** Convert to HSV and extract brightness channel. HSV(Hue Saturation Value) extract the **V-channel** (frameV = frame1(:,:,3)) to focus on intensity variations, which are crucial for detecting moving objects against the background*

   ***à** Compute background model and variance. Determine regions with significant motion by comparing pixel intensity changes over time*

   à** Identify foreground objects using thresholding. Objects are detected based on **intensity deviation from the background

   Two conditions are checked:

   - ***Brighter than background** → image > (backgroundM + thresholdFactor * backgroundVar).*
   - ***Darker than background** → image < (backgroundM - thresholdFactor * backgroundVar).*

2. **Blob Analysis & Tracking -** *Detect fish objects, extract object properties: **Centroid (x, y)**: Position of the object. **Bounding Box**: Rectangle enclosing the object. **Major & Minor Axis Lengths**: Shape dimensions. Initialize or update tracking objects.*

   - Assign detections to existing tracks.

   - Compute the required metrics: position, orientation, area, distance, and speed

3. **Extract *Aggregating Metrics Over Frames***

   *- The extracted metrics are summed over multiple frames to compute **average values**.*

Therefore, using STEPS (1), we analyzed fish behaviour changes by tracking their movement before and after a stress period from the extracted metrics. We established a baseline from pre-stress movement patterns to assess recovery and excluded data during the stress phase. Post-stress behaviour was examined in **1-minute intervals**, and each interval was compared to the baseline using a **two-sample T-test**. Once the statistical test showed **no significant difference** between post-stress and baseline movement, we identified that the fish had returned to normal. Finally, we determined

the **time taken for recovery**, providing insight into behavioural adaptation after stress. The sample processing view on the CSIC tank video using Step 1 is shown in Figure 16.

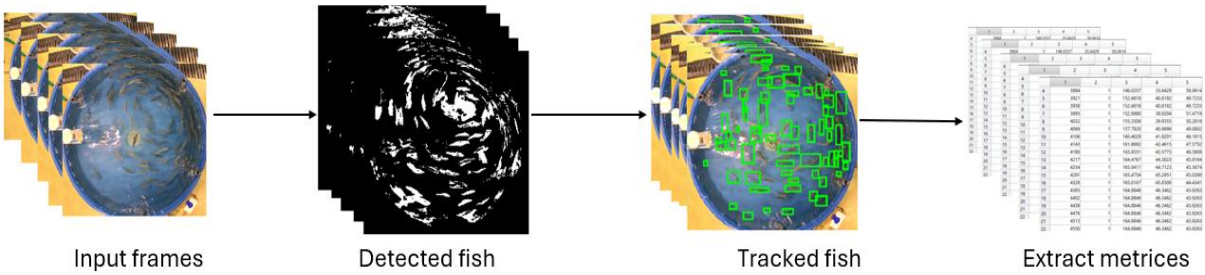


Figure 16. *CSIC video data processing using Step 1.*

HCMR - Tank

To investigate the effect of stress on fish behaviour, we employed a two-level analysis approach. First, we examined the characteristics of the acute stress response occurring on the day of the stress event. Second, we assessed the short-term response and recovery of the fish over a one-month period.

To assess the acute response of the group to stress and determine the time required for the fish to return to their usual distribution, we measured the area covered by the fish at each time point. Immediately after experiencing stress, the fish tended to aggregate at the bottom of the tank, making them less visible. Gradually, they returned to their normal behaviour, predominantly occupying the upper area of the tank (Figure 17).

To quantify this behaviour, we first processed the images by removing the background using the GMM/KNN background subtractor from the OpenCV library (Itseez, 2015), which implements the K-nearest neighbours' background subtraction method described by Zivkovic and Van Der Heijden (2006). This method also detected as foreground any significant background motion caused by sudden light variations or irrelevant movements such as air bubbles or organic particles. We applied morphological operations to eliminate these falsely detected foreground elements and retain only the actual fish (see Fig. 16).

As the cylindroconical tank used in this study was dark-coloured (i.e., black walls resulting in low-contrast images; see Fig. 16), fish near the surface were more easily detected. After processing the image, the area covered by the fish was proportional to the number of fish approaching the surface, indicating their preference for the upper tank region. This preference was quantified as the percentage of the image area covered by fish and was calculated by dividing the number of white pixels in the foreground image by the total number of pixels in the image. Low values indicated that most fish remained at the bottom of the tank, whereas high values signified a higher preference for the surface.

***Figure 17**. Vertical distribution after stress applied and gradual recovery.*

We applied an averaging window of 100 data points and Savitzky-Golay filter window of 2001 data points to remove extreme values from the data. During the stress experiment, there were some extremely abnormal values. We removed them and interpolated them using a simple linear interpolation method (in Python). We fitted our signal using a polynomial function to find the time that the fish remained at the bottom of the tank and the time needed for recovery. After exploring a range of degrees, we decided to use a polynomial degree = 30. Once we fitted the polynomial function, we calculated its derivative function. The derivative goes to 0 at the maxima and minima of the polynomial function. Therefore, we automatically detected the time points where the polynomial function reaches the maxima and minima. Then, we selected the maxima and minima around the handling period to define the experimental duration. Within that interval, we found the original signal's minimum and maximum. The time of the period in which these maxima and minima were detected was defined as the recovery time the fish needed to return to the tank surface.

H**CMR – Sea cage**

We used YOLOv5 (Jocher et al., 2022) in Python (Van Rossum and Drake, 1995; version 3.9) to detect the fish in the videos (Figure 18) after training the model with 1,000 annotated images of individual fish of European seabass. To associate each detected fish between frames, i.e., to track the fish, we applied the DEEPSORT algorithm (Wojke et al., 2017), excluding the appearance-based association parameter. All training and video analysis was performed on a Desktop computer with the following specifications: Intel Core i7-8700 3.2 GHz CPU, 32GB RAM and NVIDIA GeForce 3060Ti GPU. The fish's speed (as body lengths/sec) was calculated using the extracted trajectories.

During and immediately after the crowding event, the algorithm could not detect a lot of fish, as it could not easily detect individual fish and therefore we did not use this analysis to study the acute response. Instead, we collected data on the *speed* of the fish on D-1 (the day before), D-Day (the day of the experiment), D+1 (the day after), with a 12-day interval between the two trials, meaning from 2nd November to 4th November 2022 and from 16th of November to 18th of November 2022. For the analysis we also selected additional days to compare with the days of stress. The available data

included the speed of the fish, the time (day and hour), and the treatment (stress state = treated, or not = control). All the data were visualized using Python.

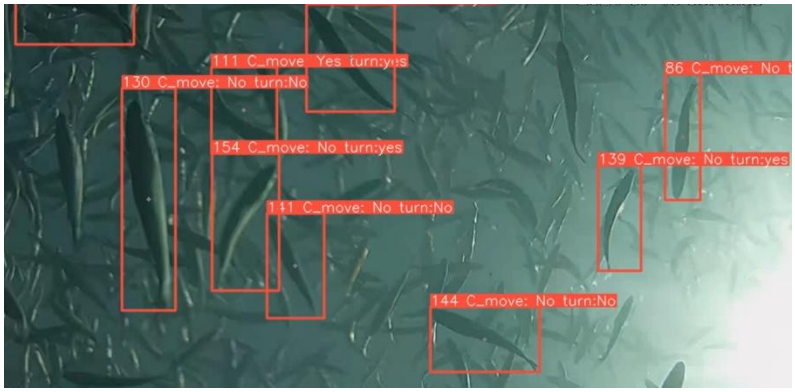


Figure 18. *An example of individual identification and tracking in sea cages.*

CSIC - Tank

The same approach was used and the same parameters were calculated as for NOFIMA video data using the classical image processing method. No CNN based approach was used.

SINTEF – Sea cage

A data processing pipeline was made to process the video data acquired from the fish pen. The processing pipeline starts by taking the video data and processes it through a neural network for detecting the fish, from the coordinate data (x,y, in pixels), we are able to generate a swimming trace timeseries for each individual fish using an object tracking algorithm. The swimming traces get stored in a snapshot file so that the heavy processing of detection and tracking only needs to be done once. From these timelines the behaviour parameters (velocity, pattern, etc..) get extracted for further statistics plotting. See Fig. 19.

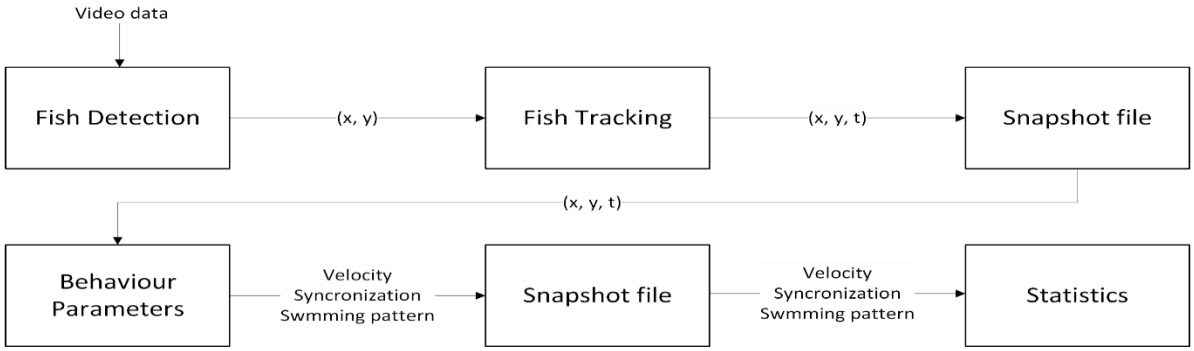


Figure 19 *A flowchart visualizing the processing pipeline for extracting the swimming behaviour from the raw video data.*

Data processing was run in three phases, (i) detection and tracking, (ii) behaviour extraction and (iii) statistics. A snapshot of the output data from each phase was saved so that parameter tweaking could be done to the next phases without re-running the time-consuming detection- and tracking. In total 325 hours of video have been processed from the confinement experiment using this pipeline.

Fish Detection

The detection is based on DDR-Net (Hong et al, 2021), a semantic segmentation neural network. The neural network was trained on a total of 836 images, where 50% were images of Atlantic Salmon from SINTEF's ACE facility at Tristeinen and 50% were images of European Seabass from HCMR's facility. Since the neural network is semantic segmentation it can only output a segmentation mask of the same size as the input image, hence, the neural network was trained to draw a circle on each fish in the video, see Figure 20.

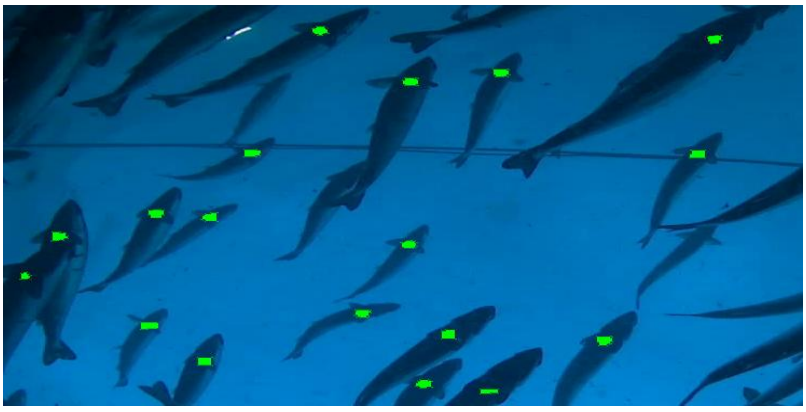


Figure 20. *A video frame with the detections (green) from DDR-net rendered on top of the raw image*

Using classical machine vision techniques, we can extract the x- and y-coordinates of each dot (which represents the position of the fish). This was done using OpenCV-function "findContours", which is an

algorithm used to find the location of contours in a binary mask or segmentation mask, like the output of DDR Net.

Fish Tracking

The fish detections from the neural network are used to map out the swimming pattern for each individual fish. This is done by taking the individual fish detections (in x,y) and run them through Norfair (Alori et al, 2023); Norfair will sort each detection with the previous detection and, based on statistics, pair the detection up with the previous one that has the highest probability of being from the same fish. Doing this we get the swimming trajectory of each individual fish, see Figure 21.

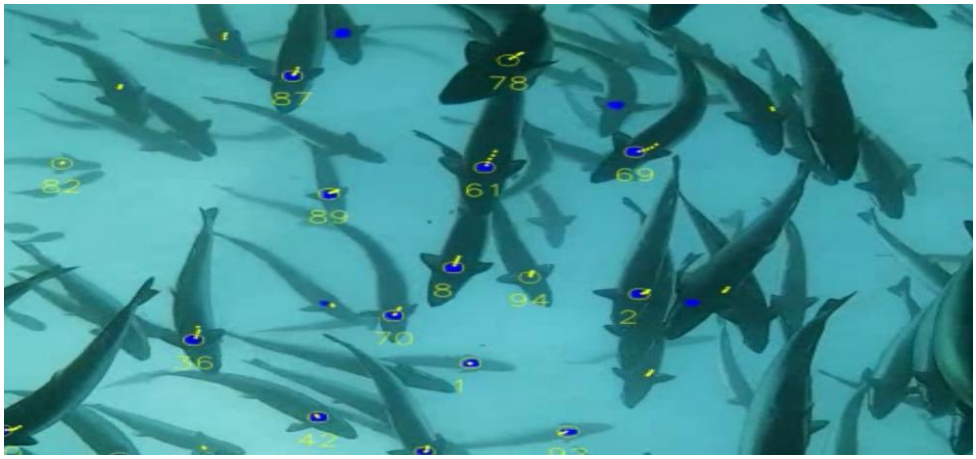


Figure 21. *A video frame with fish detections (blue), tracking IDs and previous position (yellow dots).*

The detection and tracking are done in one go during processing; for each video processed, the fish trajectory data is stored in a file as a timeseries, for easy access and modification of the post processing scrips without the need for re-processing the video data.

Behaviour parameters

From the swimming trajectory for each individual fish, a set of behavioural parameters is automatically calculated and stored as a timeseries in separate files, for quick post-processing and data plotting. The trajectory is defined as the list of fish positions $p_n(t)$, where p is the position with x and y coordinates, n is the number of fish and t is the time when the position was detected.

Swimming velocity

The average swimming speed, $\overline{v}$, of the fish population can be useful if the fish gets gradually fatigued, and swimming speed slows down over time. Additionally, the individual swimming speed, $\overline{v}_n(t)$, can indicate e.g., a deceased fish with no movement at all.

$$v_n(t) = \frac{\| p_n(t) - p_n(t-1) \|}{\Delta t}$$

$$\overline{v}(t) = \frac{\sum_{n=0}^{n} v_n(t)}{n}$$

Where Δt is the time between each frame in the video, in this case 1/25 (25 FPS), 0.04s.

Swimming pattern

At normal behavior the fish will swim against the water current along the wall of the fish pen. We can set a predetermined sliding window of 1 second (25 video frames), and calculate the average swimming pattern, $\overline{\rho}(t)$, of the population in this window.

$$\overline{\rho}(t) = \frac{\sum_{n=0}^{n} \left(\frac{\sum_{T=1}^{25} \| p_n(t-T) - p_n(t-1-T) \|}{\| p_n(t) - p_n(t-25) \|} - 1 \right)}{n}$$

When $\overline{\rho}(t)$ is close to zero it will indicate that the average fish population is swimming in a straight line (normal behavior), if $\overline{\rho}(t)$ is closer to one, it will indicate an erratic and unpredictable swimming pattern that might correlate to stress (Ciani et al, 2024).

Schooling behavior

When salmon are behaving normally, the fish population will swim in approximately the same velocity and the same direction. We can assume that the camera is placed in such a way that only a part of the fish pen is covered by the camera field of view, therefore all the fish in the population will have an almost identical velocity vector at normal behavior. By calculating the fish population average velocity vector, $\overline{V}$, we can compare this to the velocity vector for each individual fish. We can defined $\overline{s}(t)$ as the description of schooling behaviour at the specific time t.

$$\overline{V}(t) = \frac{\sum_{n=0}^{n} \frac{p_n(t) - p_n(t-1)}{\Delta t}}{n}$$

$$\overline{s}(t) = \frac{\left| \left| \sum_{n=0}^{n} \frac{p_n(t) - p_n(t-1)}{\Delta t} - \overline{V}(t) \right| \right|}{n}$$

When $\overline{s}$-value is close to zero it will indicate that the whole population is swimming roughly in the same direction (normal behavior), a $\overline{s}$-value higher than zero will indicate that the fish population is deviating from schooling behavior, this could e.g., indicate feeding or stress.

Data Processing Plan

Video data was collected from 04.04.23 to 19.05.23, the data from the following times per day was processed with the video processing pipeline in order to extract the behavior parameters, Table 3.

Table 3. *Plans for data collected from 04.04.23 to 19.05.23*

Feeding	First feeding of the day, 08:00 – 10:00
Morning	07:00 – Feeding start
Noon	One hour, start after feeding stop
Evening	17:00 – 18:00

Within this, the following times were omitted from processing due to the camera lens occlusion by a lumpsucker cleaner fish, Table 4.

Table 4. *Time and dates of the collected data.*

Date	Start time	Stop time
02.04.2023	11:01:00	12:00:00
03.04.2023	09:00:00	09:30:00
03.04.2023	17:00:00	18:00:00
04.04.2023	16:30:00	18:00:00
05.04.2023	16:21:00	16:38:00
10.04.2023	07:15:00	08:00:00
16.04.2023	17:00:00	18:00:00
17.04.2023	07:00:00	07:30:00
17.04.2023	08:00:00	08:48:00
17.04.2023	15:21:00	16:16:00
18.04.2023	08:09:00	08:30:00
18.04.2023	09:30:00	10:42:00
18.04.2023	11:44:00	12:10:00
18.04.2023	14:19:00	17:47:00
19.04.2023	00:00:00	08:30:00
19.04.2023	08:30:00	09:30:00
20.04.2023	09:00:00	10:30:00
29.04.2023	17:29:00	18:00:00
07.05.2023	17:12:00	17:49:00
08.05.2023	07:00:00	08:00:00
08.05.2023	17:00:00	18:00:00

3.4. Tags

Nofima

Information theory arose from statistics and cybernetics. The concept of information itself is based on Shannon's measure, sometimes called Information Entropy S: Where p(x) is the probability function of the investigated phenomenon x.

In the case of fish telemetry data, the measured data already represents variable distribution in time, and there is no restriction to applying entropy measurement of the available amount of information.

The Entropy is calculated per fish acceleration data, representing the average acceleration for all axes. The calculation was done for one hour using the sliding window over the signal. More details about the data processing can be found in Aquaexcel3.0 Deliverable 4.3 and Urban 2023. After calculating the entropy for both fish in the tank (in each tank, two fish were tagged by the acoustic tags), the time intervals where the difference between the entropy values was smaller than 0.2 were identified. These intervals correspond to the synchronized fish activity. These intervals were then filtered based on the interval's minimal duration (20 minutes) to remove the noise. Finally, only the intervals with the mean acceleration for both fish higher than 70 were kept because the fish activity increased during the crowding event.

The information concept itself is additive, and the probabilities are logarithmic. Therefore, the tails of the distribution and rare events are becoming more important. Information entropy could be considered a measure of surprise or a measure of our ignorance of the system. These are good predispositions to use in detecting a typical behaviour from the fish telemetry.

CSIC

The AEFishBIT device recorded acceleration in X and Y axes to measure physical activity, while Z-axis movements were used to determine respiratory frequency. Raw data from the device were pre-processed using proprietary software, filtering out noise and extracting relevant movement patterns. The data were then analyzed to determine changes in activity levels and respiratory responses before, during, and after stress exposure. The respiratory frequency was expressed as breaths/s, and physical activity in relative units, calculated as described in Martos-Sitcha et al., 2019, Frontiers in Physiology 10:667; doi:10.3389/fphys.2019.00667. It was previously determined in a swim tunnel respirometer in gilthead sea bream and sea bass that the physical activity index correlated with water speed, and operculum breathing with measurements of oxygen consumption

3.5. Sonar

SINTEF

Echosounder data in sea-cages are often affected by a shadowing effect caused by the high density of fish (Eilertsen et al.2021). To address this issue, it is common practice to either integrate data from the entire water column or identify the depth with the highest fish density. This depth can then be used in two ways: either by assuming that only the data between this point and the echosounder is reliable, or by using the depth itself as an indicator of where the majority of the fish are located. Both of these approaches have been utilised here, in addition to using the depth to section the data into an above and below said point. EchoView (13.1) and MATLAB (R2024b) were used in the processing.

Volume backscatter processing (Sv) – cleaning Sv-data

To clean and format the echosounder raw data into a more user-friendly format, it was first processed using EchoView EchoView (13.1). Once cleaned, the data was exported for further analysis using MATLAB (R2024b). The data processing in EchoView involved two main steps: The first step was to reduce the data size of the volume backscatter data (Sv), which indicates the amount of fish present. The second step was to define the line indicating the depth of the highest density. This was accomplished using the cleaned Sv-Data.

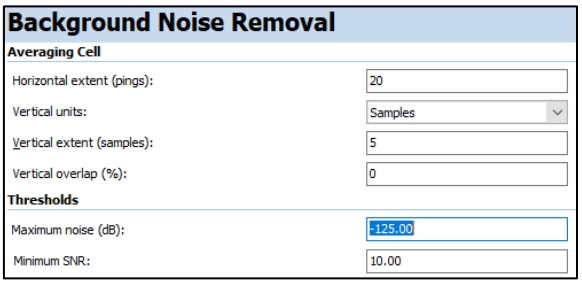


Figure 22: Settings used in the background noise removal

Raw data was imported to EchoView, before background noise was removed using EchoView's inbuilt function "Background Noise Removal". The settings used for this process are shown in Fig. 22. The data was then integrated using a predefined grid to reduce the amount of data points and then exported. To reduce data magnitude, Sv values were integrated using a grid of 1m by 10 min. Fig. 22 shows the raw data with noise removed. The overlying raster indicates the size of the cells which were integrated over and used in further analysis.

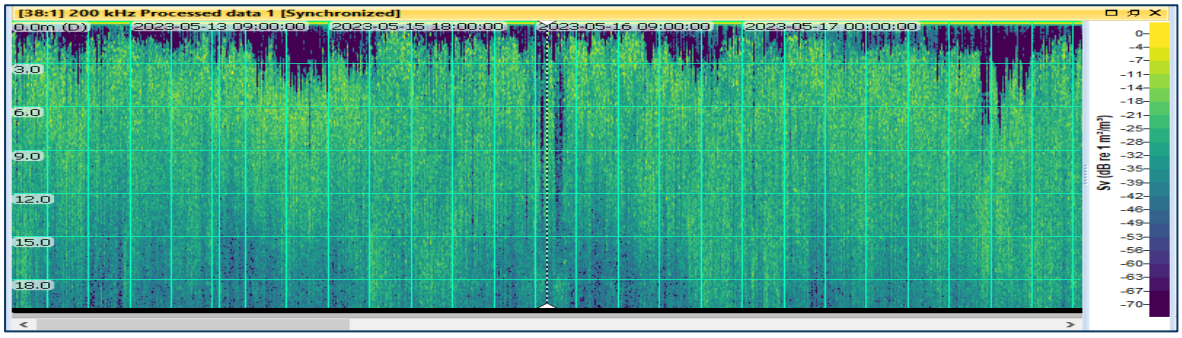


Figure 23: *The raw data after noise removal with grid showing the size of cells used for integration.*

Line processing – Finding depth of maximum Sv

The approach for finding the maximum Sv-Line involves additional steps beyond the background noise removal used on the Sv raw data. The first step is to resample the data with a lower time interval of 10 minutes. This resampled linear data is then multiplied by two to increase the difference between weak and strong echoes. Amplifying the difference between the echoes ensures that the inbuilt line detector algorithms in EchoView works more efficiently.

The lines of interest in the data are the top and bottom of the fish school, as well as the point of highest Sv value in the column. Fig. 24 shows an example of data where the portions above and below the lines indicating the fish school have been removed. To detect the top and bottom of the fish school, the inbuilt "Best Bottom Candidate Line Pick" was used, either searching from the bottom of the echogram (for bottom of school) or from the top of the echogram (for top of school). After the region containing the fish school has been defined, the point of highest density is identified by using the inbuilt function "Maximum Sv Line Pick".

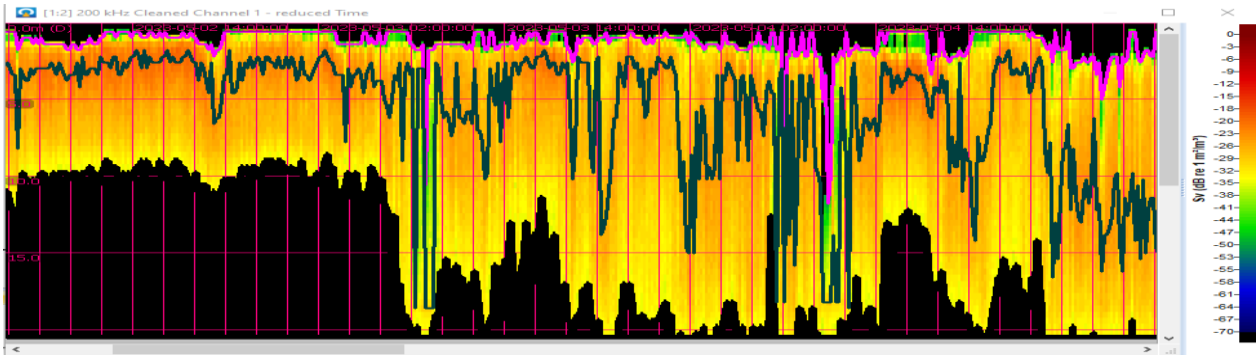


Figure 24. *Example of the Sv-data with the area above the top of the school (pink line) and below the school (black line) removed. The grey-green line indicates the depth of maximum Sv within the school.*

MATLAB processing: Normalizing the data – assumptions

The exported data from EchoView was then imported to MATLAB for further analysis. Due to lack of in-depth calibration of the echosounders, comparison of the recorded values was challenging. Therefore, the following assumptions were made to allow comparison of data between echosounders:

1) On average each echosounder detected the same amount of fish over the study period.
2) We can normalize the data by finding the average Sv value over the entire cage depth, and over a sufficiently long period (in this case approximately 1 month of data)

To find the normalized values, the average Sv-value for each echosounder had to be identified. This was done by taking the mean linear Sv-value over relevant depths (cell 2-20) and over the entire period. The results for the four echosounders, presented as dB, are shown in **Table 5**. The integrated Sv-values could then be normalized using the Channels average Sv values and thereafter compared with each other.

Table 5*: Average volume backscatter values over cell 2-20 for the entire period of data collection showcasing how there is a relatively small difference between the channels, indicating that the assumption that they see similar amount of fish during the study is supported.*

	Channel 1	Channel 2	Channel 3	Channel 4
Cell 2-20 mean Sv in [dB]	-24.75	-24.64	-25.33	-25.46

Comparing echosounder data

Once the Sv-data had been cleaned and the values normalized, the echosounder data could be processed for comparison. The first cell of each echogram was excluded due to noise, and then a subset of the entire water column was chosen, specifically the top 14.5m due to the fact that once the cage was crowded the outer echosounders would potentially not have any fish beyond this depth. To compare the echosounder data, three different approaches were applied. The first approach was to summarize the data within the chosen range, while the second was to calculate the mean. The third approach introduced a new assumption that the amount of fish visible at any time in all of the

echosounders should be the same, that is: the sum of all echoes in all four normalized echosounder data should be 1. As all three approaches showed similar trends, only the third approach will be included here.

To ensure that there were no shadowing effects, the depth of highest volume backscatter was identified and used to divide the data into an “above depth of strongest echo” and a “below depth of strongest echo”. The mean of each of these two subset was calculated and used to compare echosounder data.

The goal of these comparisons was to see if the fish were moving in a certain pattern during crowding compared to “baseline” conditions. ***First***, the normalized values were compared between echosounders placed in the centre of the cage (Channel 2 + 3) and those along the cage net (Ch. 1 + 4). ***Second***, the data from the echosounders on one side of the cage (Ch. 1 + 2 = left) was compared with the ones on the other side (Ch. 3 + 4 = right). It is important to keep in mind that the number of cells included in each subset varies with the position of maximum depth.

4. Results

4.1. Cages

4.2. HCMR

The speed variation was similar during the period of study and outside the stress trial periods (no-stress days), showing only slight variations (Fig. 25). Initially, and before any trial started, the average speed was 0.51 ±0.13 bd/sec (body length per second). Between the two trials the speed was 0.52 ± 0.11 bd/sec and after the second trial it was 0.51 ± 0.12 bd/sec.

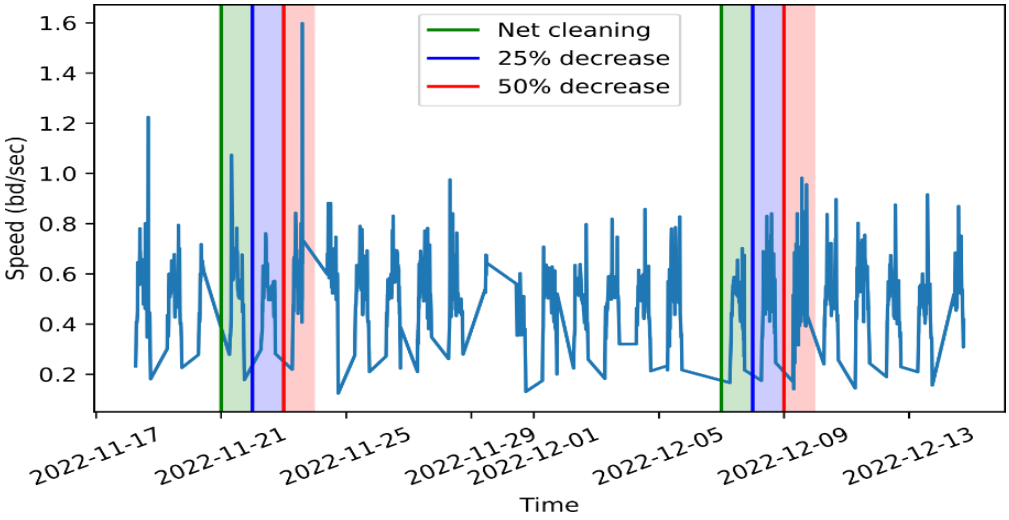


Figure 25. *Speed variation for different days. The colored shades indicate the day of each stress event. Green: Net cleaning, Blue: 25% decrease, Red: 50% decrease.*

During the first trial (from 21st to 23rd of November) the speed appeared higher than in the no-stress days (0.56 0.15 bd/sec). However, the average speed during the second trial was significantly lower than in the first trial and closer to the speed of no-stress days (0.49 0.12 bd/sec). Within the day, we see that the highest speed difference between trial 1 and trial 2 is observed mainly in the morning and afternoon (Figure 26 left). The daily pattern of activity and the values of speed remained the same across the no-stress periods suggesting that the speed was not affected by any of the two trial events (Figure 26 right). A difference in speed was observed when we grouped the period after the first trial into two sub-periods. Immediately after the first trial (from 24th to 29th of November) the speed was higher 0.54 0.11 bd/sec than that of the later days, i.e. before the second trial (0.50 0.10 bd/sec).

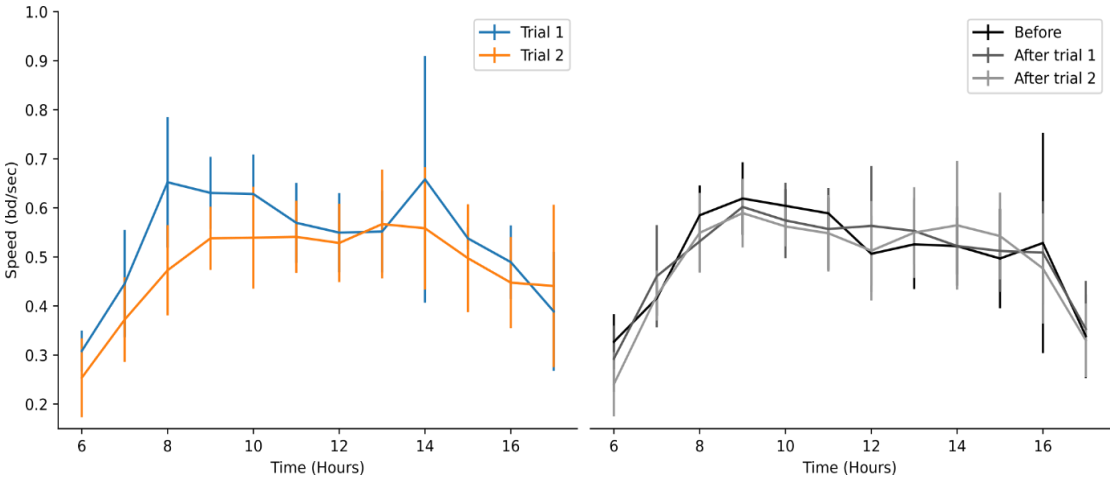


Figure 26. *Daily variation of speed (average and standard deviation) of for the different trials (left) and no-stress periods.*

Focusing on the trial periods, we observe that the effect on the speed depends on the type of stressor applied but also the trial order (Figure 27). More specifically, we see that net cleaning resulted in higher speed (0.55 0.15 bd/sec) when the stressor applied for first time and the speed remained higher even after the stressor applied, i.e. across the whole day. In addition, the daily pattern of activity was different with fish showing a stronger peak in the activity in the morning and in the afternoon. During the second trial the speed was lower (0.46 0.11 bd/sec).

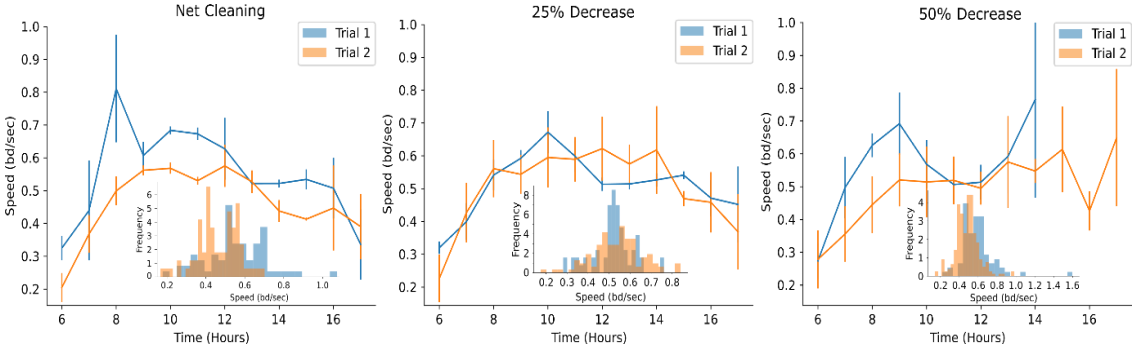


Figure 27. *Daily variation of the speed and the respective histogram of the speed for the different stressors (shown in the three different subplots) and the different trials (shown in different colors).*

Decreasing the volume of the cage to 25% did not show any effect on the activity pattern or the average speed values, and this was found at both trials (0.52 $\pm$ 0.10 bd/sec and 0.53 $\pm$ 0.12 bd/sec). In contrast, the decrease of cage volume to 50% of the initial had an effect on the activity pattern and the speed values. When the stressor applied for first time the average speed was significantly higher (0.60 $\pm$ 0.18 bd/sec) than the speed found during the no-stress days but also during the net-cleaning

event. In addition, the daily activity show a stronger peak in the morning. When the stressor applied for second time, the speed was lower (0.48 $\pm$ 0.12 bd/sec) than in the first time.
Regarding the environmental parameters, the temperature dropped approximately 2 ^{o}C between the trials but remained similar during the stress events of trial 1 (20.54 $\pm$ 0.01 ^{o}C) and trial 2 (18.8 $\pm$ 0.15). The oxygen levels were stable during the experimental period (5 $\pm$ 0.30 mg/L) and where not affected after applying any of the stressors or at any trial.

4.3. SINTEF

Sea cage video data
The video data was processed according to the Data Processing Plan. **Erreur ! Source du renvoi introuvable.**8 illustrates the behaviour parameters timeseries along with wind data acquired from the closest weather station, at Halten Fyr.

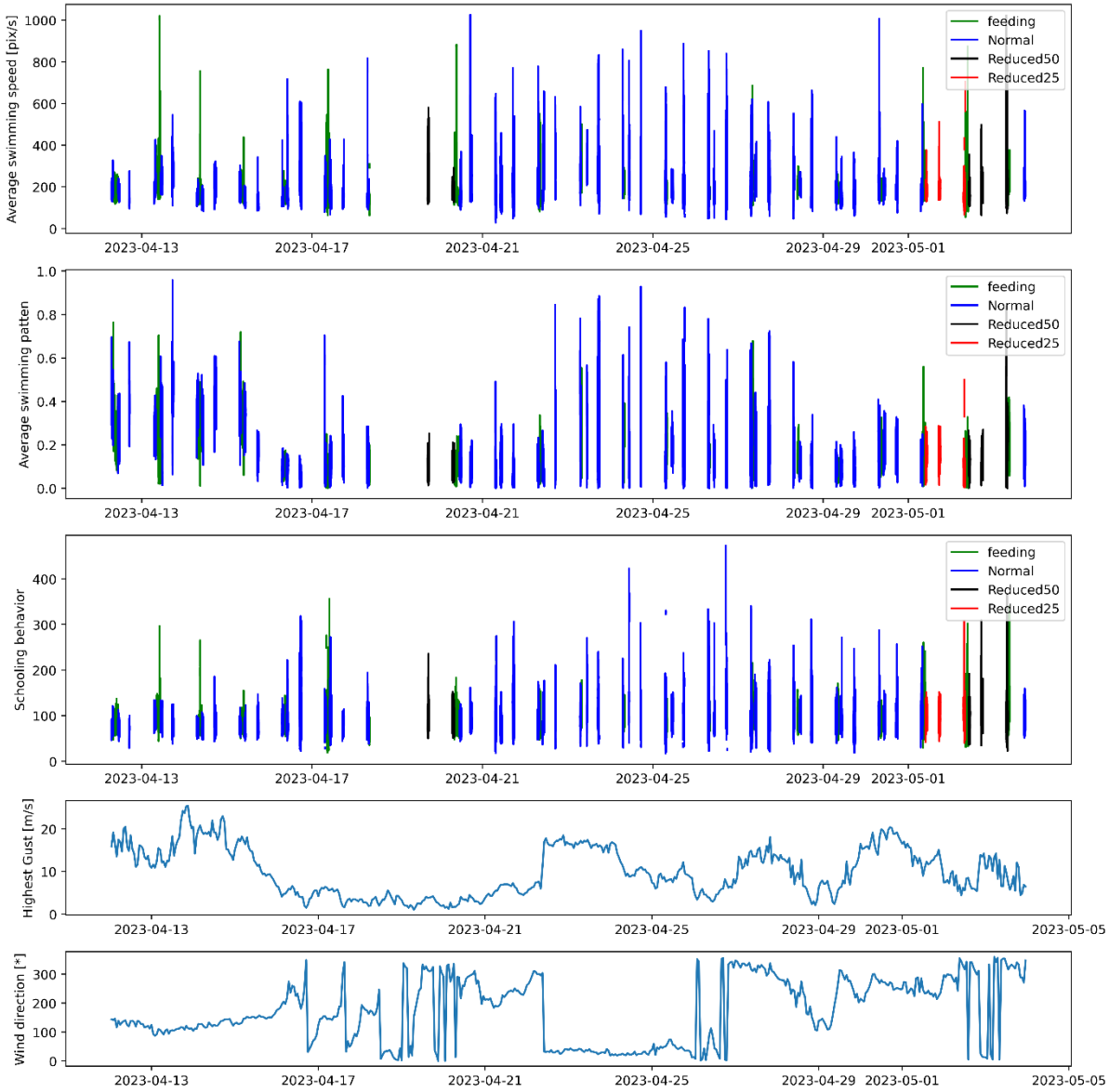


Figure 28. *a plot illustrating the average swimming velocity (subplot 1), average swimming pattern (subplot 2), schooling behaviour (subplot 3) and wind speed- and direction (subplot 4 and 5).*

Initial assessment of the data indicated no correlation for the calculated behaviour parameters to the time of confinement, but rather to the wind speed. Thus, more detailed investigation of the fish behaviour was split into before, during and after confinement.

Pre-confinement

As a baseline for "normal" behaviour, we plotted the average swimming vector in a wind rose for each day, to look at the average swimming direction and velocity the six days before confinement, seen in

Figure 29. The dominating swimming direction is to NW and SE on April 12th and April 13th, and a weight towards NW at 14th and 15th, with a slight weight back towards SE on the 17th. There is no specific pattern, both swimming direction- and velocity is different from day to day.

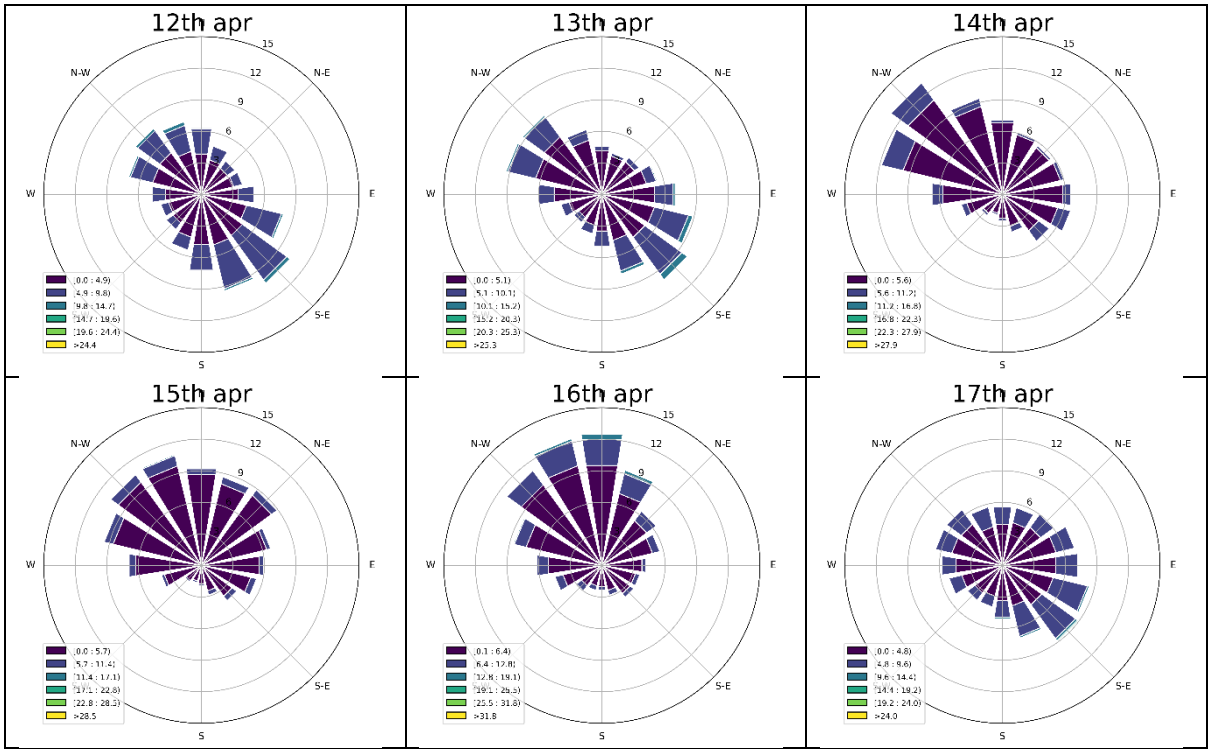


Figure 29. *A plot illustrating the average swimming velocity and direction per day, six days before first confinement (April 18th).*

Erreur ! Source du renvoi introuvable.30 shows that there is a significant change in the swimming pattern from 14th to 16th, this is most likely due to high winds on the 13th, calming down around the 16th (Figure timeseries plot), this could also explain the increase in schooling behaviour around the same date.

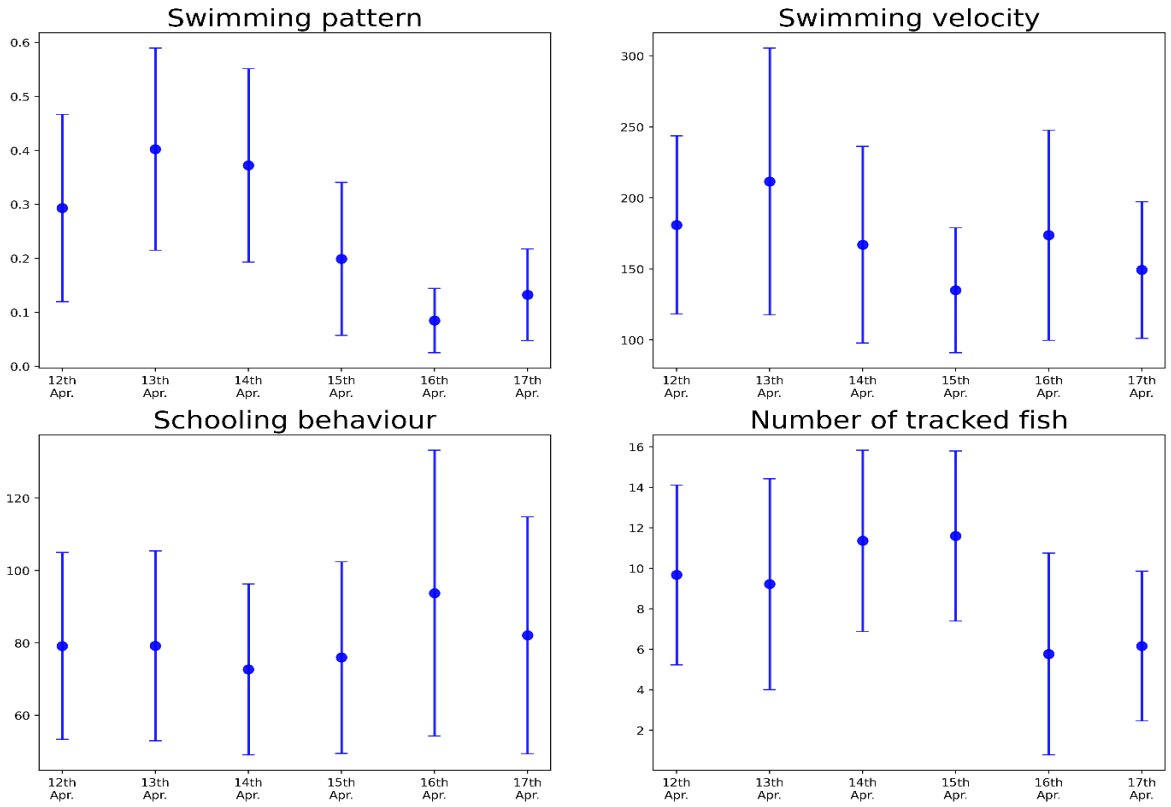
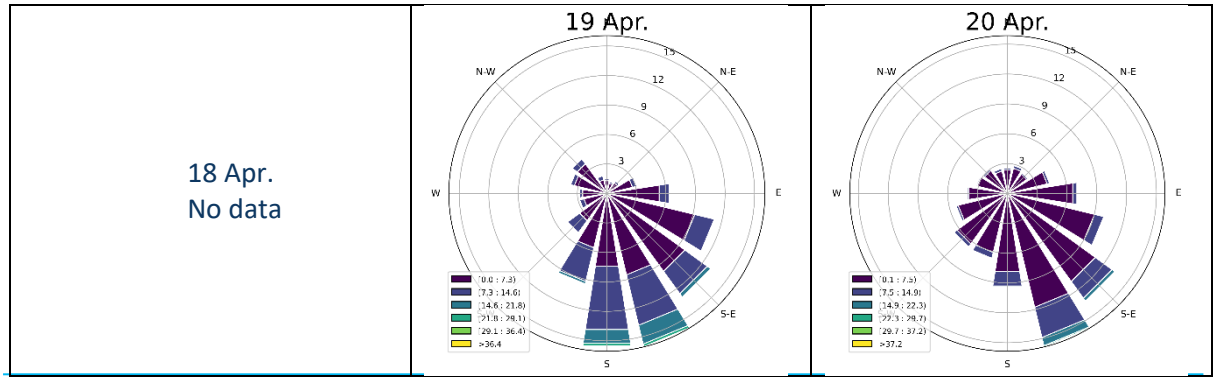


Figure *30. A plot illustrating the behaviour parameters (subplot 1 to 3) plus the average consecutive number of tracked fish (subplot 4) per day, six days before confinement.*

Confinement

When looking at the data (**Erreur ! Source du renvoi introuvable.**31) we see that for the first confinement (19th) and the day after the fish is swimming mostly towards S – SE. While during the second confinement the fish are swimming mostly towards W – SW. The lack of data on the 18th is due to the camera occlusion of a lumpsucker fish the entire duration of that day. The data suggests that there are no distinguishable changes in swimming direction or –velocity, during- or after confinement.



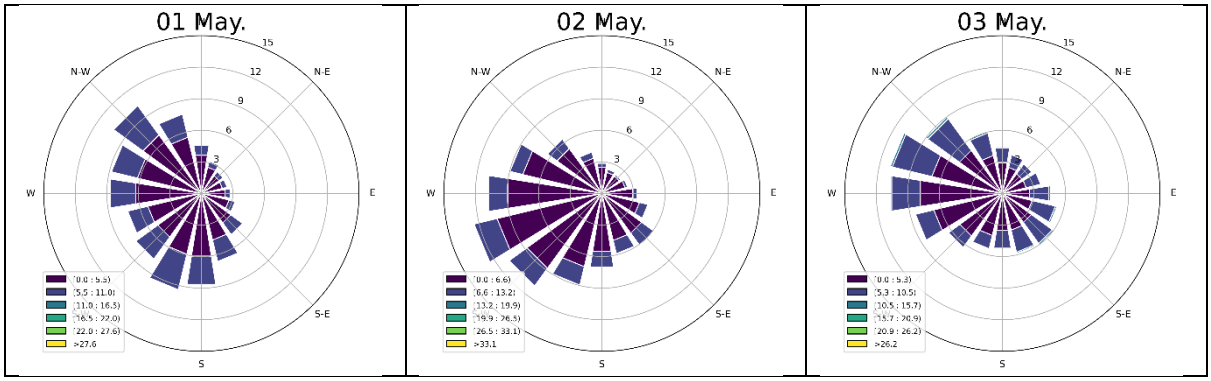


Figure 31. *A plot illustrating the average swimming velocity and direction per day, for 1st confinement (18th- and 19th of April) plus the day after 1st confinement (20th of April). And 2nd confinement (1st- and 2nd of May) plus the day after 2nd confinement (3rd of May)*

Erreur ! Source du renvoi introuvable.32 suggests that there are no significant changes in behaviour during- or after confinement for both trials. The swimming pattern parameter are slightly lower for the first confinement, but not significantly lower.

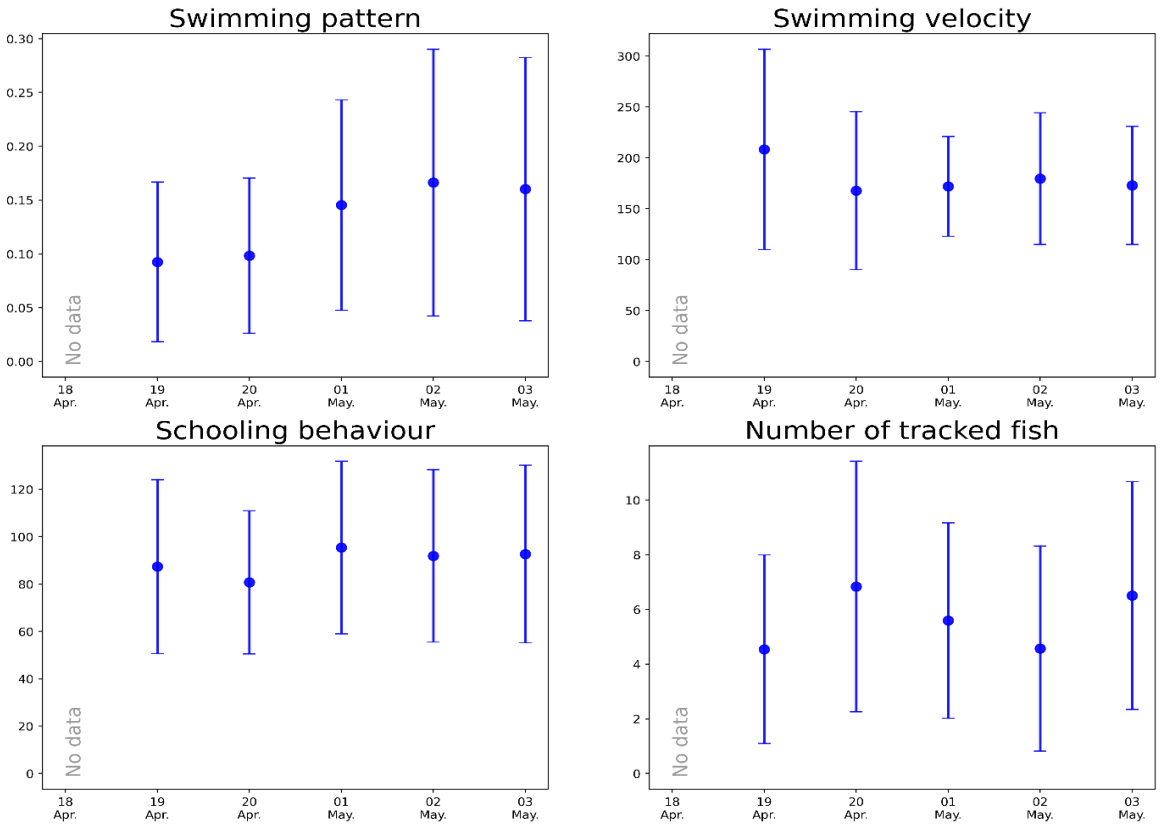


Figure 32. *A plot illustrating the behaviour parameters (subplot 1 to 3) plus the number of tracked fish (subplot 4) per day, during the first- and second confinement.*

Summary

When looking at the average swimming direction- and velocity Figure 33, we see that the swimming direction is gradually changing counterclockwise from W – NW to S – SE with a constant swimming velocity during the next four days. However, the gradual change does not suggest that confinement had an impact on swimming direction- or velocity.

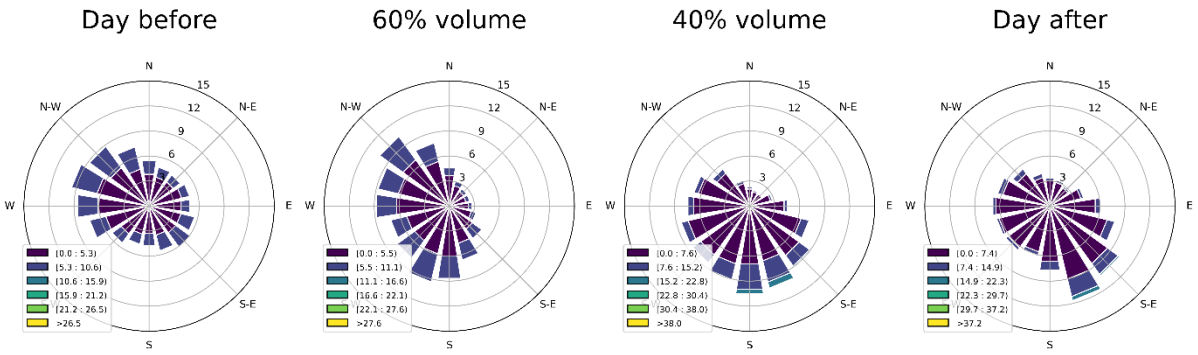


Figure 33. *Illustrating the average swimming direction and –speed before, during and after both confinement trials*

When illustrating the fish behaviour for both confinement trials it becomes clear that there is no significant change in swimming behaviour during or after the confinement, see Figure 34.

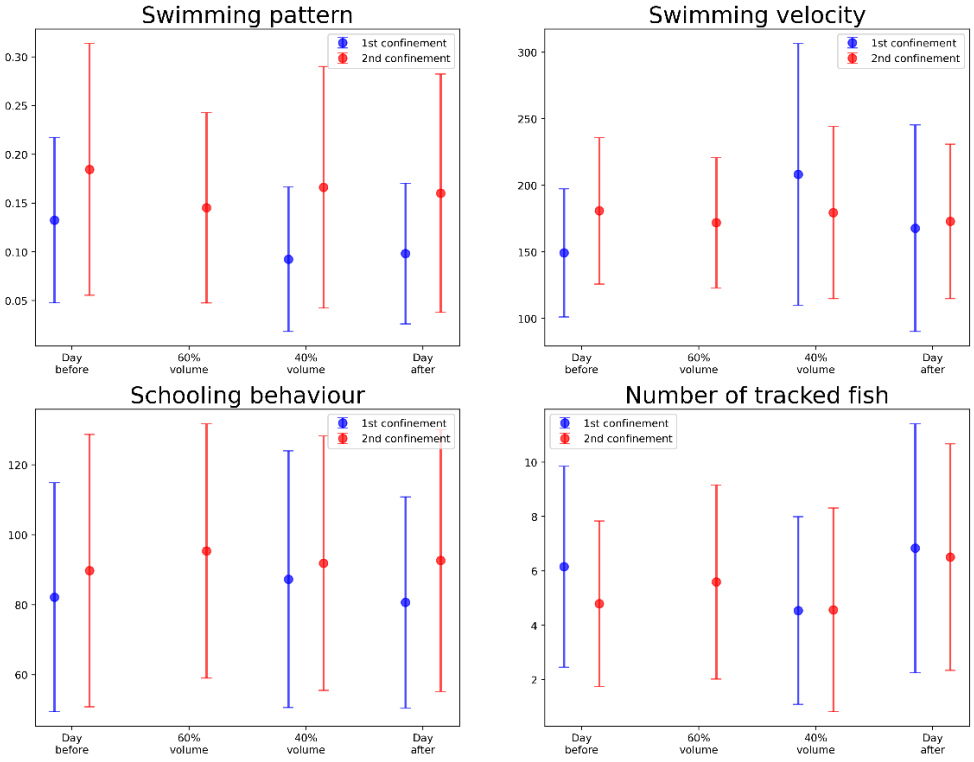


Figure 34. *illustrating the fish behaviour (subplot 1 to 3) and the number of fish tracked (subplot 4) for first trial (blue) and second trial (red), before-, during- and after confinement.*

Echosounder

Baseline behaviour

Fig. 35 shows the “Depth of maximum Sv” for the entire study period. Note the two periods where data is missing around 23rd of April and 14th of May. To showcase a baseline for the fish before studying the effect of the crowding, the period from the 8th of April to 15th of April is shown in more details in Fig. 36 as an example of baseline behaviour.

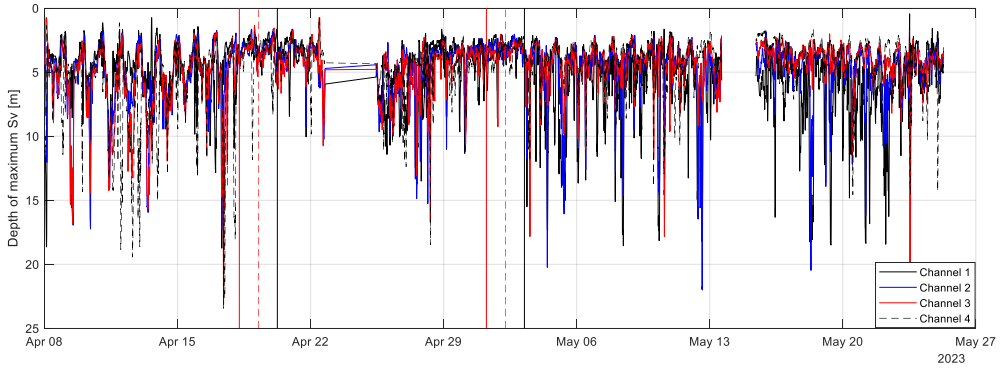


Figure 35. *Depth of maximum Sv in each channel for the entire period. The vertical red line indicates when crowding was started, the dotted red line when crowding was intensified and the vertical black line when crowding ended.*

Baseline data shows a relatively similar echo in the four channels, with the “Depth of maximum Sv” having relatively concurrent fluctuations in the channels Figure 35(B), and relative similar values in all channels for the “Comparative amount of fish” Figure 35(A). Furthermore, there are no clear trends of the fish favouring one side or the other, or the centre or the outer region, except a weak tendency for more fish in the centre of the cage after midnight Figure 35(D). This indicates an equal distribution of the fish in both depth and horizontal distribution within the cage volume when undisturbed.
There are, however, shorter periods of spikes indicating an asymmetry in the fish distribution in the cage, for instance on the 9th of April. Here the “weighted comparison of Sv” indicated that there was more fish in Channel 4 Figure 35(A), while the “depth of maximum Sv” Figure 35(B) indicated that the fish in Channel 1 and 2 during the same period swam deeper. It is uncertain what caused this response.

Figure 36. *Each figure shows the period prior to crowding, giving an indication of baseline behaviour. A) shows the weighted comparison of normalized Sv from 1.5 to 14.5m. B) Shows the depth of maximum Sv in [m]. C) Shows the mean above the depth given by B), and is used as a basis for the plots in D) that show the comparison of left and right, and center and outer echosounder data.*

Crowding behaviour

During both crowding operations, the "depth of maximum Sv" is unsurprisingly at a higher depth (Figure 37 (B) and (F)). There is also an increase in "Mean above max-Sv" compared to baseline (Figure 37 (C) and (G)) compared with Figure 37(C), i.e. more fish is observed in the upper water column. In addition, there is less variation between channels in the "comparative amount of fish" compared to baseline (Figure 37(A) and (E)), i.e. fish were comparatively more consistent in their use of space in the cage.

Comparison of fish distribution in the echograms did not show distinct trends that were consistent for both crowding operations (Figure 37(D) and (H)). During the first crowding, fish was observed swimming closer to the net walls of the cage (Figure 37D)). During the second crowding, the fish were keeping more to the left of the cage but were more evenly distributed between the centre and the outer region of the cage (Figure 37(H)). During intensification of crowding 2, the fish appeared to "flee" from the right side of the net pen.

While the number of fish was slightly higher in the upper 14.5 m during both crowding events, this trend was not very distinct.

Behaviour after crowding differed after the two operations. After the first crowding, the fish continued to congregate closer to the surface (Figure 37(B)). After the second crowding, the fish spread out more (Figure 37(F)), and two distinct peaks indicating a sudden change in behaviour were observed. It is uncertain what caused this sudden change in behaviour, but similar behaviour was observed in connection to net cleaning operations.

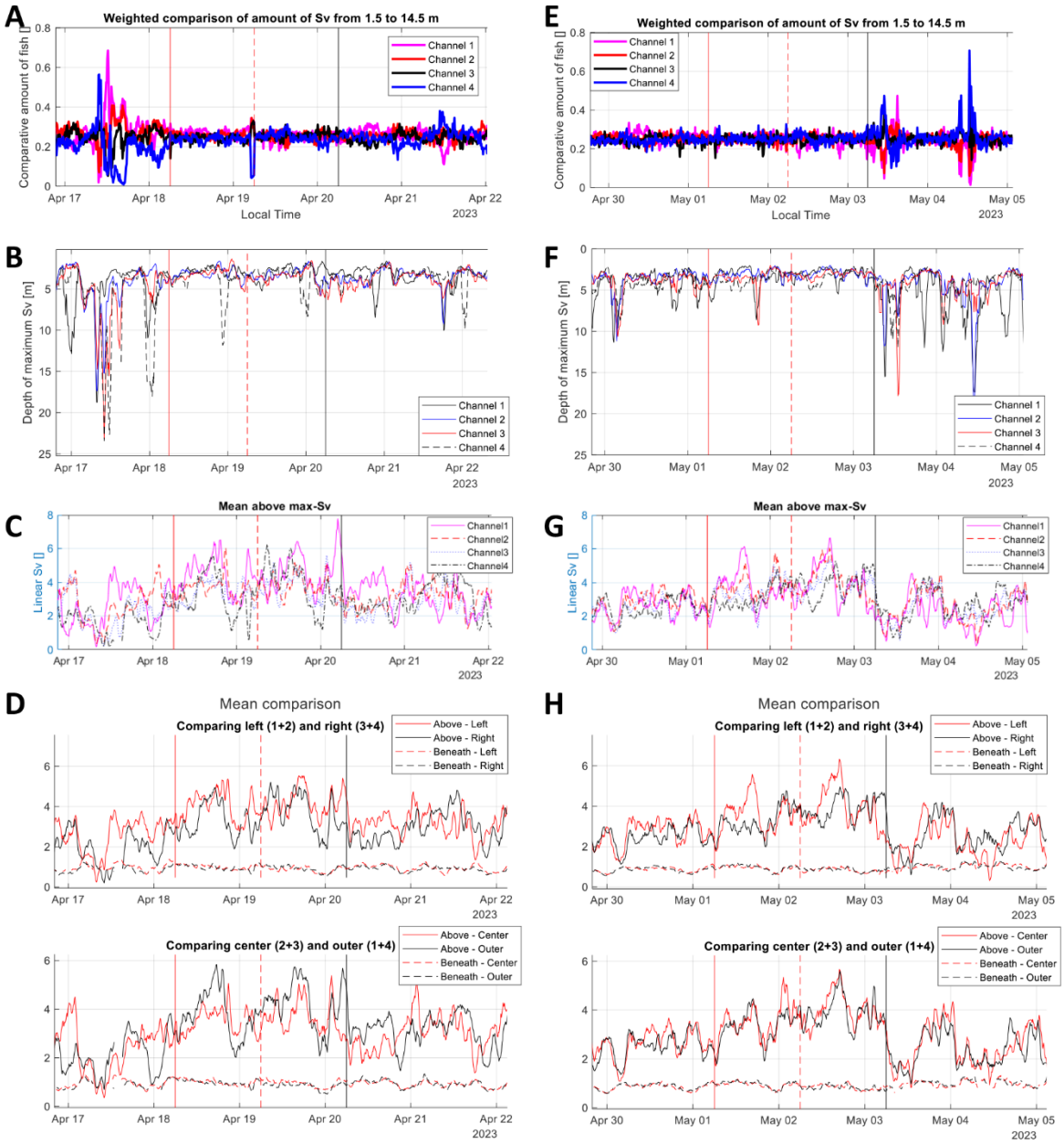


Figure 37. *Close up of the two crowding periods, with the first crowding shown in graphs A-D and the second crowding in graphs E-H. The vertical red line indicates when crowding was started, the dotted red line when crowding was intensified and the vertical black line when crowding ended. A and E show the depth of maximum Sv in all channels, while B and F shows the Sv-data analysed using the depth of maximum Sv-data used to calculate the mean of the subset above shown in C and G. D and H show the comparison of the data in C and G, but combined into groups of left and right and centre and outer. Data in all figures has been smoothed using a 5-step moving mean window.*

Net cleaning activities

As described above, the fish were typically making use of the entire cage volume, indicated by similar echosounder signals from the four channels. However, during net cleaning, deviation from this pattern was observed. The cage was cleaned on the 17th of April, 28th of April and 10th of May. During these periods the fish appear to avoid either Channel 1 or Channel 4, which are the channels closest to the cage net (Figure 38). This is particularly clear on the 17th of April where the fish first avoided Channel 1, before later avoiding Channel 4 (Figure 37). On the 10th of May and the 28th of April, avoidance concentrated on Channel 1 Figure 38(B).

In addition to these net cleaning operations there are also other periods showing such abrupt avoidance, for instance 12th of May. It is uncertain what might have caused a similar reaction this day, as there was no cleaning activity registered on this day.

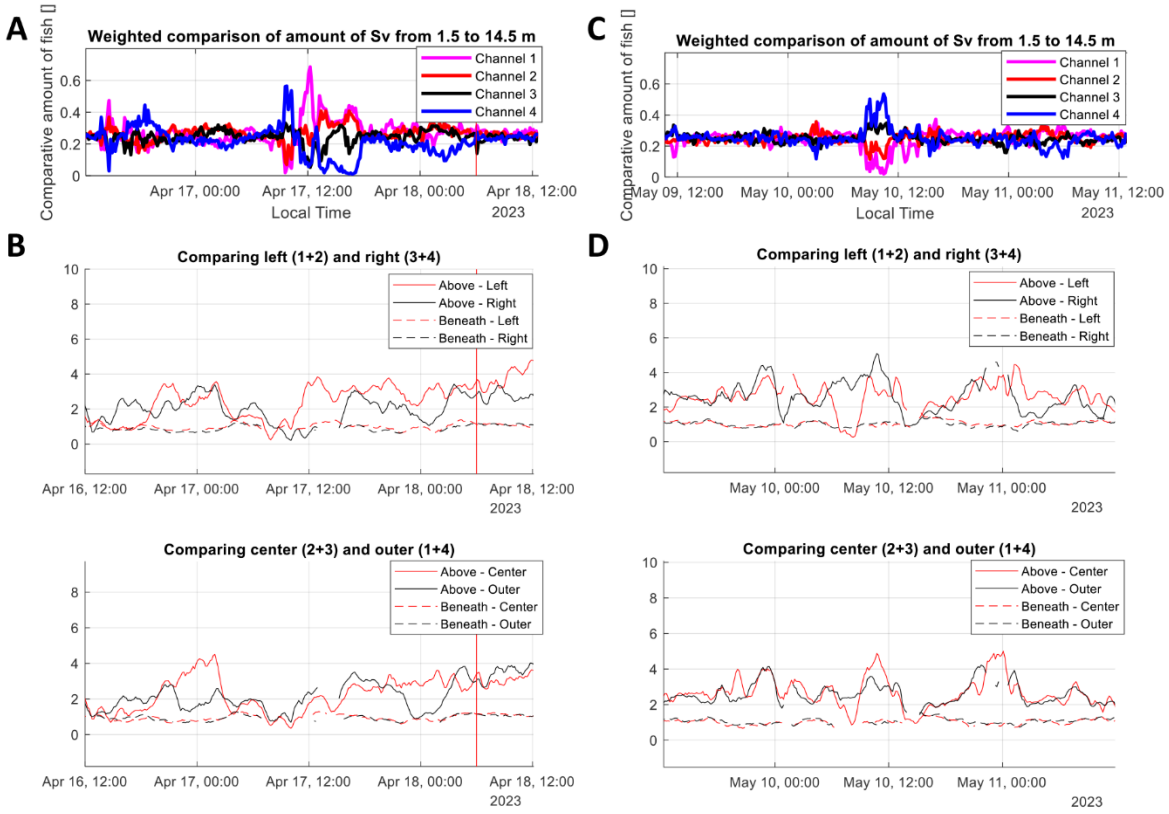


Figure 38. *Examples for observations during periods with known net cleaning activity, A) and C) show the first netcleaning, B) and D) the third. On the 17th of April net cleaning was conducted from 09:00 to 11:00 UTC and on the 10th of May from 07:45 to 10:30. In both cases, an abrupt drop in signal strength is observed in some of the channels nearest the net wall (Channels 1 and 4). Note that the data Is limited to the top 14.5 meters.*

4.4. Tanks

4.5. HCMR

The dissolved oxygen (7 $\pm$ 1 mg/L) remained stable throughout the experimental period, and was not affected by the stressor.

Growth data:

There were no differences between the control and the stressed groups in terms of growth with both groups increasing in weight by 150 g by the end of the trial. Similarly, baseline cortisol levels did not differ between the groups, exhibiting stress values within the typical seasonal range for the species at approximately 200 mg/ml (Fig. 39). However, there were slight differences in conversion efficiency, with FCR increasing for both groups after the first sampling, possibly reflecting effects of sampling stress. However, by the end of the trial FCR for the control group dropped to initial levels while for the stressed group remained with, perhaps indicating long lasting effects of the repeated stress at the organismal level (Fig. 40). Moreover, the prevalence of external abnormalities differed between stressed and non-stressed groups, with the former exhibiting higher, and increasing over time, frequency of visual cues compared to the control group (Fig. 41). The majority of those cues (58%) were located to the fin and tail area while other abnormalities like missing scales and local redness were also identified.

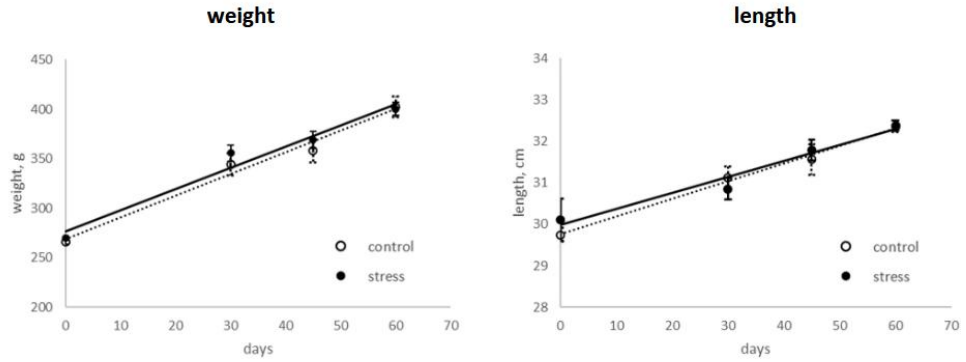


Figure 39. *Growth performance parameters (weight and length) in time for the control and the treated group.*

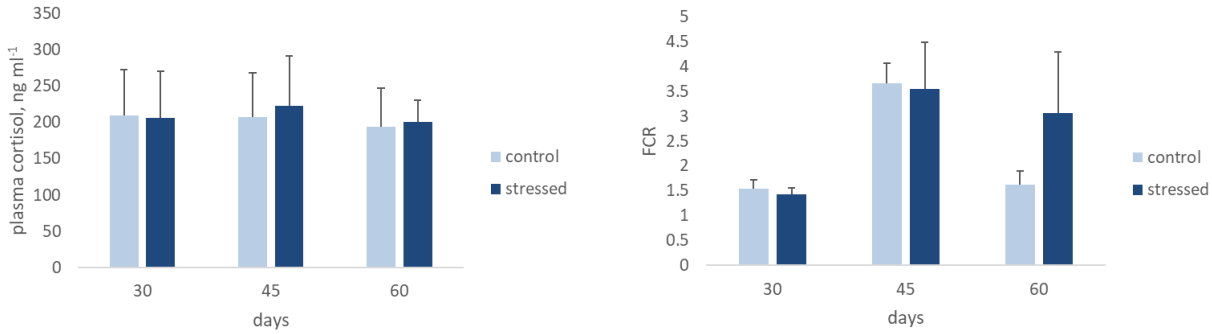


Figure 40. *Barplots of the plasma cortisol levels (left) and the FCR (right) for the control and treated group.*

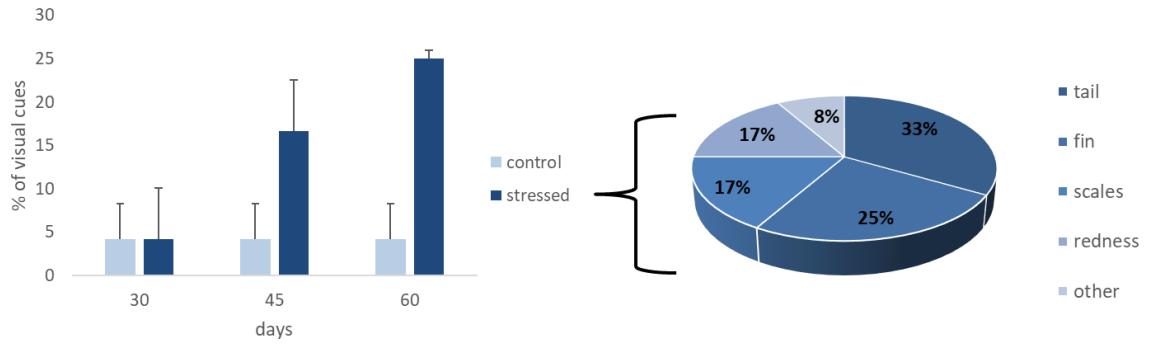


Figure 41. *Barplots of the percentage of visual cues for detecting morphological abnormalities in the control and treated group. The pie shows the percent of the specific morphological abnormalities detected.*

Acute stress response

The treated group needed a longer time (2.12 ± 1.31 hours) to return to the tank surface after the stressor applied for first time in comparison with the control (0.58 ± 0.30 hours; Table 6). When the stressor was repeated, two weeks later, the time the fish needed to return to the surface decreased (1.66 ± 0.15 hours) but remained higher than the control group (0.51 ± 0.16 hours). The recovery duration of the control group did not differ between the analyzed days.

Table 6. *Recovery duration (in hours)*

	$Duration_{min\_max}$ (Hours)
Treated	
2022-11-03	2.12 ± 1.31
2022-11-17	1.66 ± 0.15
Control	

2022-11-03	0.58 ± 0.30
2022-11-17	0.51 ± 0.16

More details on the variation of the area covered and the detected recovery duration are shown in Fig. 42. The time period the fish spent to the bottom of the cage (i.e. the percent area covered was small) depended on the time the fish were in the confined space for the treated group (30-40 mins), the duration of the blood sampling and the time there was human presence and activity around the tanks. For this reason, there is stronger variation of the duration of the fish being at the bottom in the treated group (Monitor-1 and Monitor-3 in Fig. 42) than in the control.

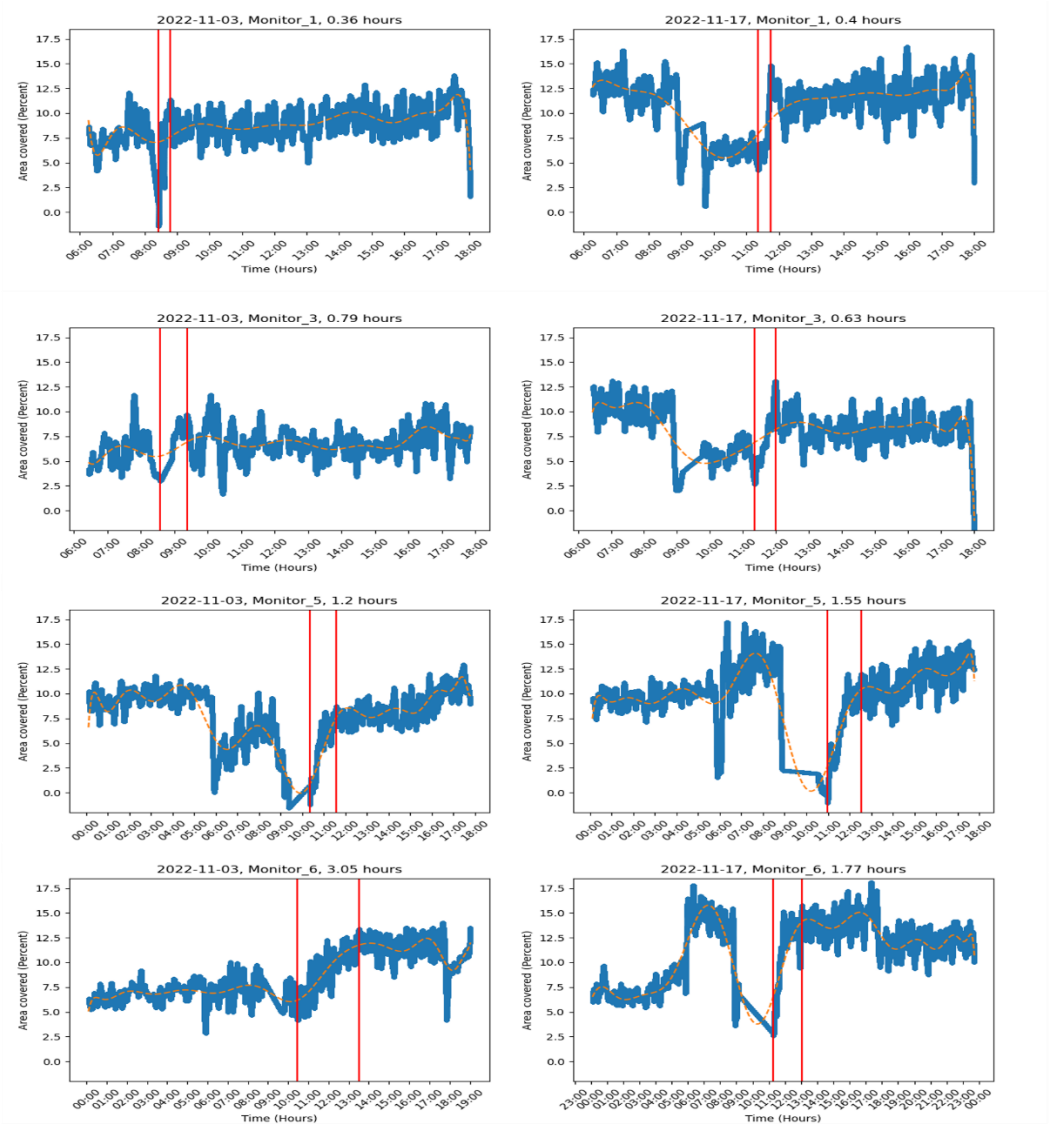


Figure 42. *Percent of the area covered by the fish for different times and dates, and for different monitors. Red vertical lines show the start and the end of the transition as defined by the minimum*

and maximum values of area. This time interval defines the recovery duration. Monitor-5 and Monitor-6 are the treated groups. Monitor-1 and Monitor-3 are the control groups.

Short-term stress response

Regarding short-term effect of behaviour, our results suggest that there are no differences in the speed caused from the stressor (Fig. 43). The treated group appeared to have a lower swimming speed than the control group, but this difference was apparent throughout the whole experimental period, even before any stressor was applied. After the stressor applied, the difference between the control and the stressed group increased slightly. This could possibly indicate a weak effect of the stressor on the behaviour of the treated group. Moreover, towards the end of the trial differences between the groups are negligible, possibly indicating habituation of the treated group to the repeated stressor.

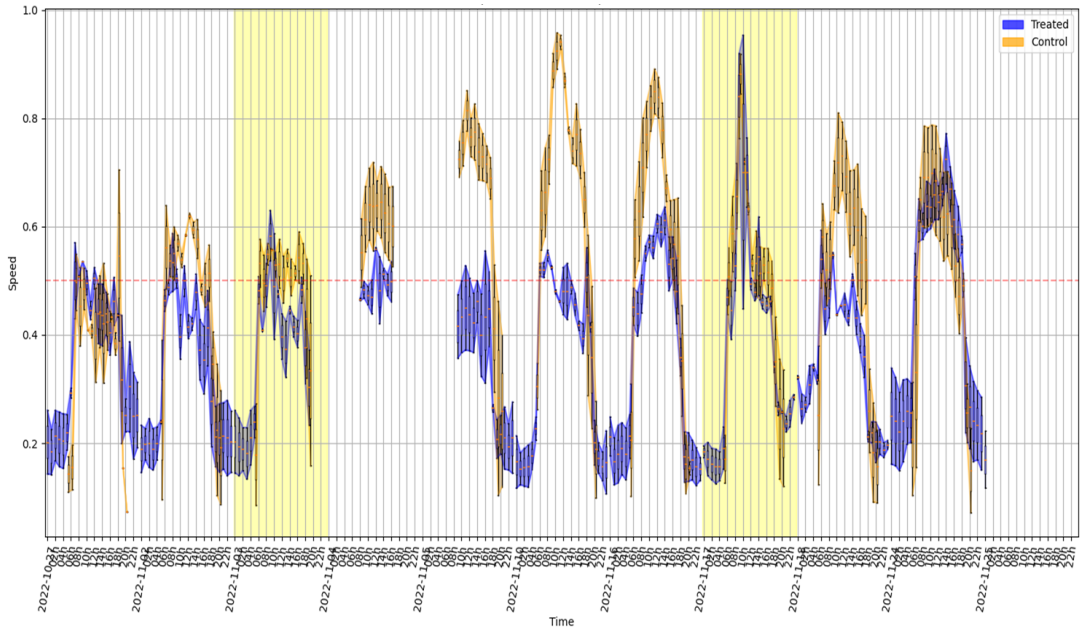


Figure 43. *Daily speed variation of the control (orange) and treated group (blue) for a period of a month.*

4.6. CSIC

Only one video which includes three distinct phases: before stress, during stress, and after stress is available, Fig. 44. Specific timestamps are provided, with stress beginning at 02:02:00 and ending at 03:09:30. Additionally, Fig. 45 presents extracted parameters from the CSIS video, including area, position, speed, orientation, and distance.

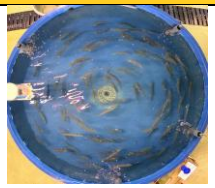
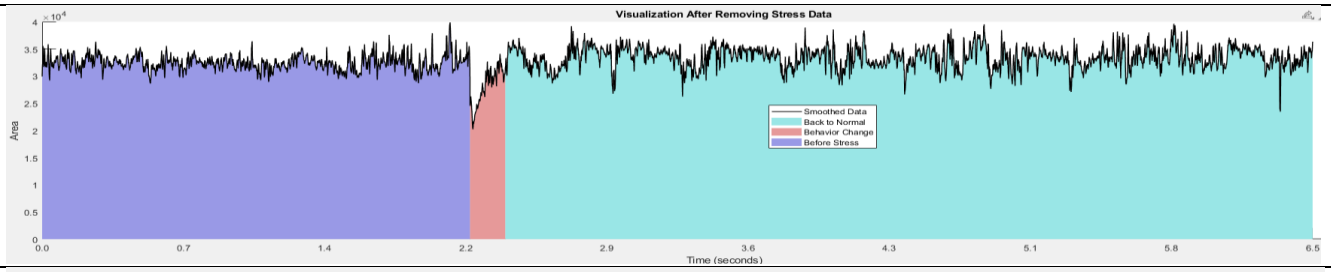
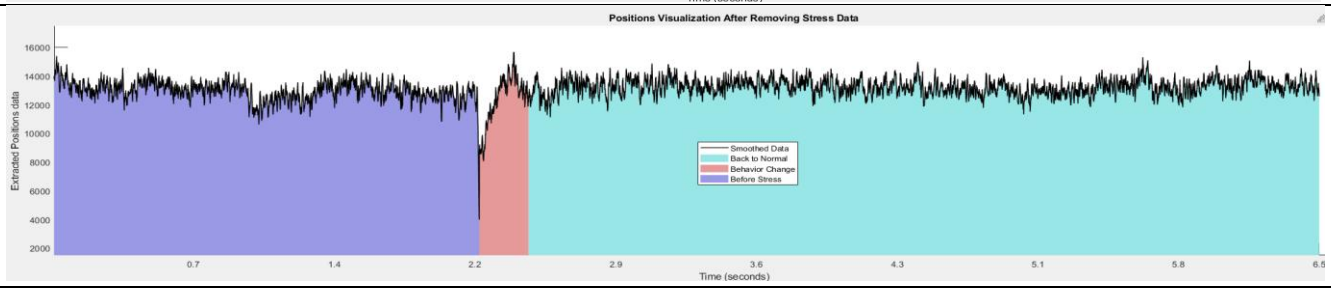
CSIS	Before stress		During stress	After net removal		Remarks
- **Total Video length is** 07:10:29	 -View before the stress	 -View 20 min before stress i.e. at -02:22:00	 -View during the stress	 -View immediate after removal of stressor	 -View 10 min after removal of stressor	-Stress stat at 02:02:00 -stress ends at 03:09:30 -extracted parameters are presented in Figure 43

Figure 44. *Overview of the CSIS video, breaking it down into key phases: Before Stress: Includes a view 20 minutes prior to stress (at -02:22:00). During Stress: Captures the stress phase from 02:02:00 to 03:09:30. After Net Removal: some view after immediate net removal, and a view after 10 minutes post-stressor(net) removal with can be seen that the fish are returning back to their normal behavior movement in the tank (accordingly with human eye point of view).*

CSIS Tank	
Area	
Position	

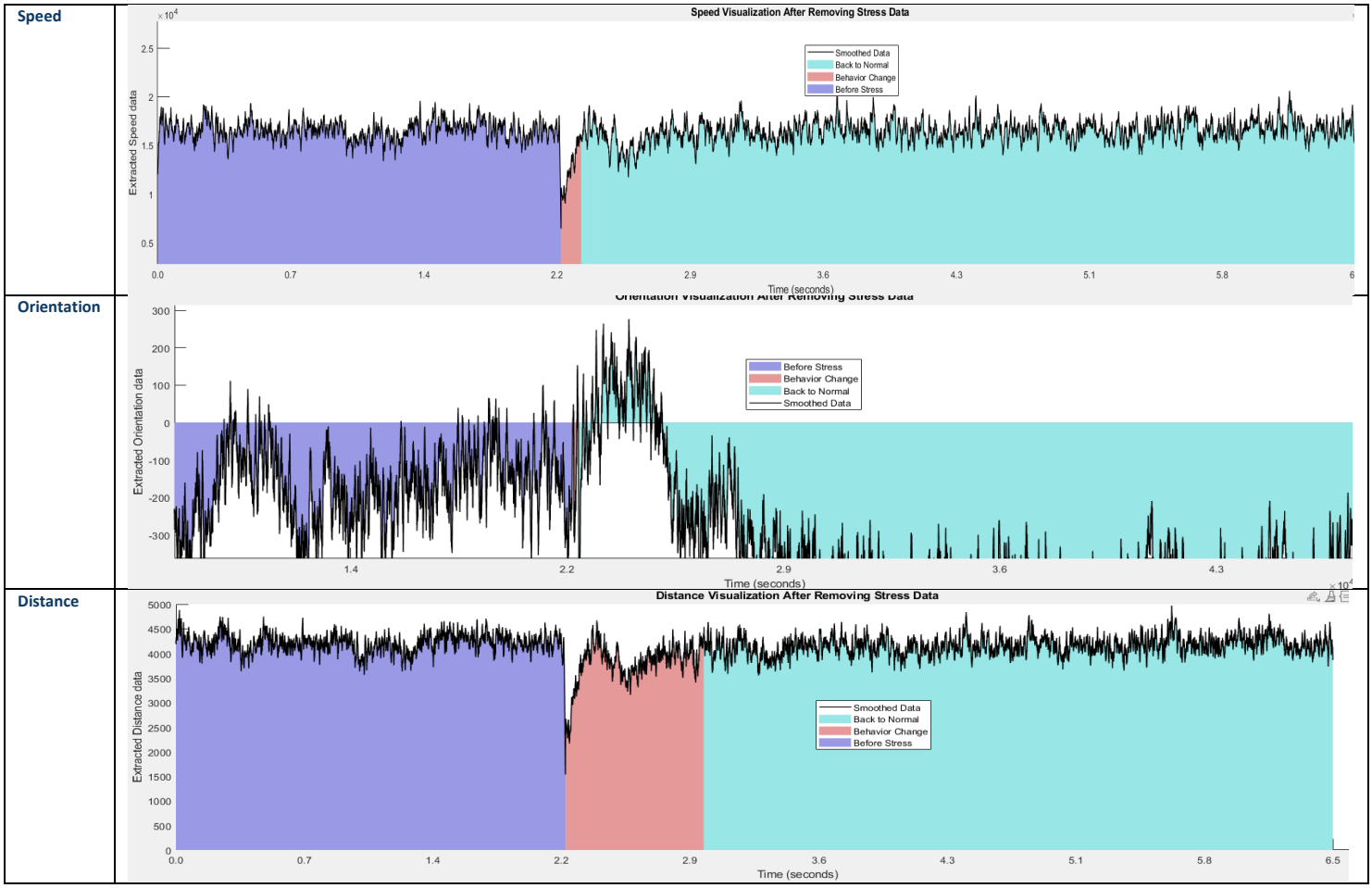


Figure 45. *Graphical Representation of extracted metrics data: This table visually represents the extracted data from the CSIS video, including Area (size occupied in the tank), Position (movement coordinates), Speed (rate of movement), Orientation (direction faced), and Distance (total distance of each fish from the tank center point)*

4.7. NOFIMA

NOFIMA video data consists of the records three tanks (108, 113, and 115). However, although in the videos data, the fish experienced significant stress at different times or repeatedly over short intervals following the removal of the net, which was the primary stressor. Additionally, other disturbances contributed to further stress, affecting the reliability of the results for these tanks. All the three tanks have recorded data from different days for each tank, i.e.

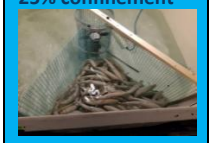
Video 1-May 19, June 3, and June 18 of 2023 as normal behaviours data.

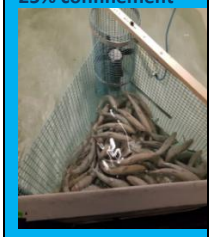
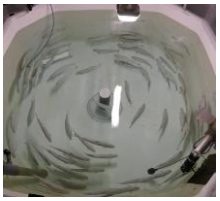
Video 2-May 24, June 8, and June 23 of 2023 were the days for confinement with 25% stress with the net in the tank and removed the stressor on the same day. Whereas, we have
Video 3-on May 27, June 11, and June 26 of 2023 are the data recorded after 2 days after the stressor, and unfortunately the fish are already recovered from primary stressor.

But, during the confinement recording we have some data which have some recording on the fish behavior data on the immediate removing of the primary stressor, though these video data are few seconds of time, and we have computed these data just to check if any behavior changes are visible.

Table 7 presents a detailed breakdown of the Tank 108 short video data sequence recorded on the specific day for better clarity and it also shows the result detailed computed or processed using the STEPS 3.2a.

Table 7. *NOFIMA tank 108 details*

Data Before Stress(Video 1)		Data after stress recovery(Video 2)			Data 2 days after stress recovery (Video 3)	
Date	**Remark**	**Date**	**Remark 1**	**Remark 2**	**Date and Tank View**	**RESULT**
19-05-2023	**Total Video Time 1:01:23**	**24-05-2023**	**Total video Time is 00:01:51**	**The Net was removed at 00:01:45, leaving us with 6 seconds of data to process.**	**27-05-2023 Total video Time is 00:48:37**	
	- Pre-Stress View 	**25% confinement** 	**-Some fish were removed during the stress phase, and water was filled after net removal until the end of data recording.**	**-View after Stress End** 	**-View in the tank** 	**-6 seconds of spike or drop in movement (refer to Table 4.1b (1))**
03-06-2023	**Total video Time 1:01:37**	**08-06-2023**	**Total video Time 00:04:03**	**The Net was removed at Time - 00:03:33. Thus, we have 00:00:30 data to process**	**11-06-2023 Total video Time is 00:48:03**	
	-- View before Stress Start  **- Minor movement or disturbance occurred during data recording**	**25% confinement** 	**-Some fish were removed during the stress phase. Water was filled both during stress and after net removal at 00:03:50 until the end of the video.**	**-View after Stress End**  **-View during water filling affter the net removal** 	**- View in the tank** 	**-Slight spikes in area observed, but all parameters appear similar before and after stress. Refer to Table 4.1c(1)**
18-06-2023	**Total video Time 1:08:01**	**23-06-2023**	**Total video Time 00:11:59**	**The Net was removed at Time - 00:04:07 Thus, we have 00:7:52 data to process**	**26-06-2023 Total video Time is 00:59:28**	

	- View before Stress Start 	25% confinement 	-Some fish were removed during the stress phase. Water was filled both during stress and after net removal until the end of the video.	-View after Stress End  -View at 00:5:28 some movement till - 00:5:56 	- View in the tank 	After removal of net or stressor, we can see slight spike in area. Refer to **Table 4.1c(1)**

In Table 7, the data is organized into three categories: observations before stress (Video 1), data after stress recovery (Video 2), and data 2 days after stress recovery (Video 3). Here's a detailed description of each category:

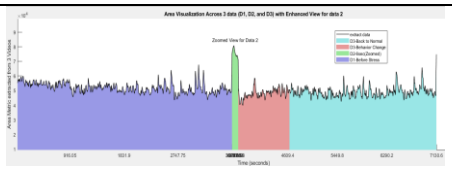
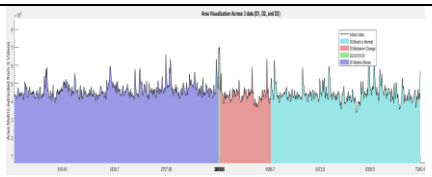
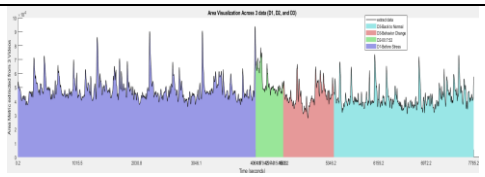
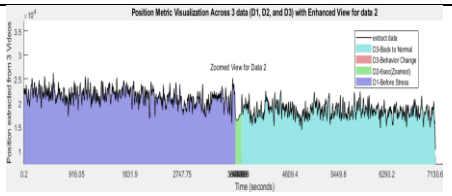
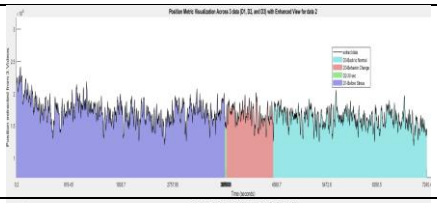
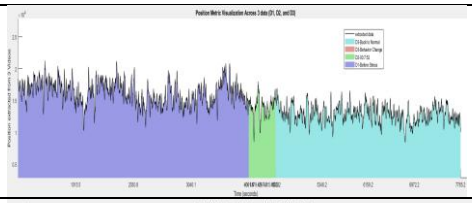
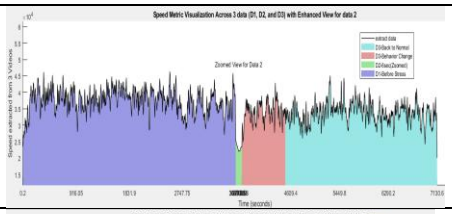
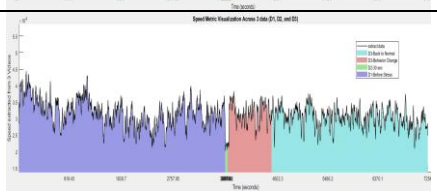
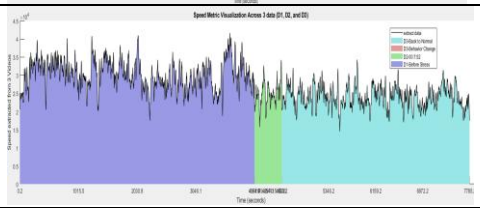
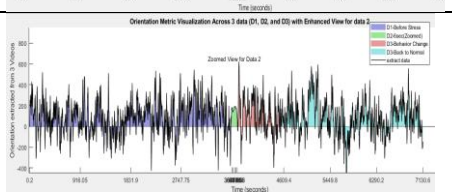
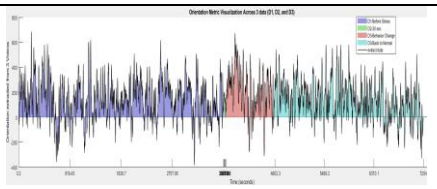
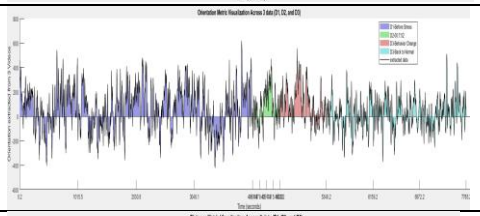
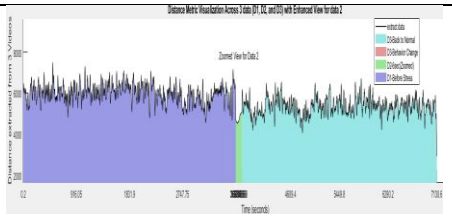
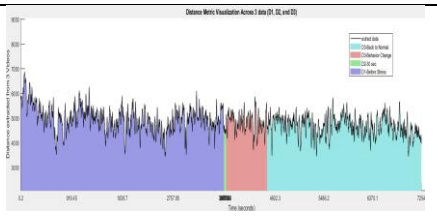
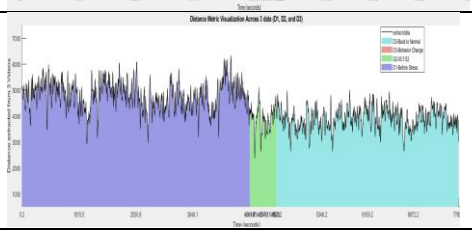
1. Data Before Stress (Video 1):
   - The recordings were made on three dates: 19-05-2023, 03-06-2023, and 18-06-2023, with total video durations of 1:01:23, 1:01:37, and 1:08:01, respectively.
   - These videos capture the conditions before any stress was applied, that provides a baseline for comparison.
2. Data after stress recovery (Video 2):
   - The observations during stress were made on 24-05-2023, 08-06-2023, and 23-06-2023, with video durations of 00:01:51, 00:04:03, and 00:11:59, respectively.
   - The stressor was removed at specific times:
     - 24-05-2023: At 00:01:45, leaving 6 seconds of data to process or analyze.
     - 08-06-2023: At 00:03:33, leaving 30 seconds of data.
     - 23-06-2023: At 00:04:07, leaving 7 minutes with 52 seconds of data.
   - Common observations shown on the table:
     - Some pictures on 25% confinement on stressed phase and net were used as a tool for stressing the fish.
     - Some fish were removed during the stress phase, and water was filled after net removal sometime until the end of data recording.
3. Data 2 days after stress recovery (Video 3):
   - Recordings were made on 27-05-2023, 11-06-2023, and 26-06-2023, and the total video time are of 00:48:37, 00:48:03 and 00:59:28, respectively.
   - These videos show the fish view in the tanks after 2 days of the stress event, capturing post-stress behavior.

Each video series corresponds to Video 1, Video 2 and Video 3 are combined and data metrices were extracted using STEPS 3.2a. The results section refers the specific graphical representation in different figures for the different parameters in Table 108, for visual reference of the extracted results.

This structured timeline and detailed observations provide a comprehensive understanding of behavioral changes associated with stress events.

Table 8 presents the graphical analysis of data collected from Tank 108 on the specific dates as mentioned in Table 7. The table focuses on five key behavioral parameters: Area, Position, Speed, Orientation, and Distance.

Table 8: *Graphical presentation of the extracted data from the videos from tank 108.*

Tank 108	Results for Data from 19-05-2023, 24-05-2023 and 27-05-2023	03-06-2023, 08-06-2023 and 11-06-2023	18-06-2023, 23-06-2023 and 26-06-2025
Area			
Position			
Speed			
Orientation			
Distance			

By comparing these metrics across the three different dates, the table offers a comprehensive view of the behavioral impact of stressors on the fish in Tank 108. Since we have very little data extracted after the stress from Video 2, which is represented as data 2 or D2 in the graphical representation, it is important to note that in column 2 we have data in only 6 seconds long, Column

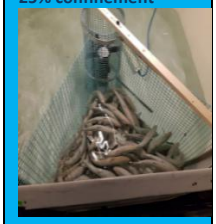
3 we have less data extracted from video 2 only 30 sec, and in column 4 we have 7:52 sec of data extracted from video 2.

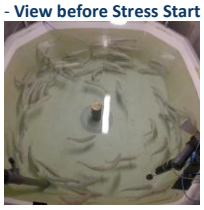
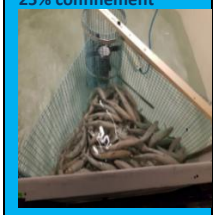
However, to better visualize the patterns, in column 2 only the D2 from Video 2 has been stretched, whereas D1 and D3 are presented in their original scale without any stretching or zooming. This approach provides a clearer perspective on the immediate behavioral changes post-stressor removal.

Note*: For the graphs in* Table 8 *column 2, to achieve zooming in our analysis, we applied a stretch factor to expand the time scale of the selected video segment. By adjusting the stretch factor (initially set to 5 and later increased to 15), we controlled the level of zoom. The time frames within the analysis period were extracted and scaled using this factor, effectively stretching the timeline to create a zoomed-in effect. This transformation ensured that the video segment appeared more detailed, allowing for closer inspection of behavioral changes over the chosen time window.*

From the graphical data visualization we can say that we don't see any differences in the extracted data from the recorded video in Tank 108. Moving towards another tank 113, we have Table 9 this presents a detailed breakdown of the Tank 113 short video data sequence recorded on the specific day for better clarity and it also shows the result detailed computed or processed using STEPS 3.2a.

Table 9. *NOFIMA tank 113 details*

Data Before Stress(Video 1)		Data after stress recovery(Video 2)			Data 2 days after stress recovery (Video 3)	
Date	Remark	Date	Remark 1	Remark 2	Date and Tank View	RESULT
19-05-2023	Total Video Time 01:01:25	24-05-2023	Total video Time 00:05:37	The net was removed at Time- 00:05:08. Thus, we have 00:00:29 data to process	27-05-2023 Total video Time 00:01:07	
	- View before Stress Start 	25% confinement 	- Some fish were removed during the stress phase, and water was filled after net removal until the end of data recording.	-View after Stress End 	- The data received for this day was not in video format but rather a stack of images -View in the tank 	After stress: -Slight spike in area -Drop in distance, speed, and position Refer to Table 4.1c(1).
03-06-2023	Total video Time 01:00:21	08-06-2023	Total video Time 00:08:43	The net was removed at Time - 00:02:28 and we have 00:05:20 data to process.	11-06-2023 Total video Time 01:05:00	
	-- View before Stress Start 	25% confinement 	- Some fish were removed during the stress phase. Water was filled both during stress and after net removal until the end of the video.	-View after Stress End  -View at 00:3:09 some movement till 00:3:23 	-View of the tank 	Unable to differentiate; all parameters appear the same before and after stress. Refer to Table 4.1c(1).

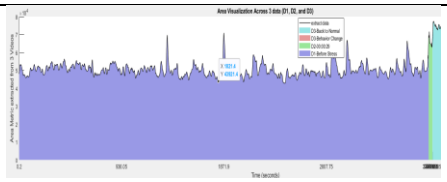
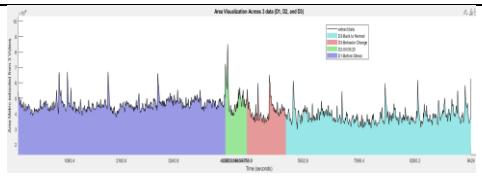
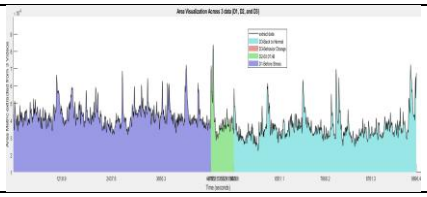
18-06-2023	Total video Time 1:08:03	23-06-2023	Total video Time 00:11:59	Stressor removed at Time - 00:04:12 Thus, we have 00:7:48 data to process	26-06-2023 Total video Time 01:02:17	
	- View before Stress Start 	25% confinement 	-Some fish were removed during the stress phase. After net removal, some unwanted movement and high-current water flow were observed until the end of the video.	-View after Stress End  -View at 00:4:45 some movement till 00:5:18 	- View in the tank 	Unable to differentiate; all parameters appear the same before and after stress. Refer to Table 4.1c(1).

Similarly, Table 10**,** represents video data collected at different time observations before stress (Video 1), data after stress recovery (Video 2), and data 2 days after stress recovery (Video 3). Here's a detailed description of each category in Tank 113. Here is a detailed description:

1. Before Stress (Video 1)
   - Recorded total video durations at different dates.
2. Data after recovery of stress (Video 2)
   - Some picture for knowing that fish were confined during stress using net (25% confinement).
   - Som fish were removed, and water was filled after net removal.
   - And we have a short segment of data available for analysis (times are noted as shown in table).
   - Some unwanted movements or disturbances were recorded after net removal.
3. Data 2 days after stress recovery (Video 3)
   - Videos were sometimes received as stacks of images instead of continuous footage.

The data metrices were extracted from these datasets using STEP 3.2a results are presented into multiple sessions in Table 10, each covering different stress events and recovery periods. Post-stress observations included slight spikes in area, drops in speed, and positional changes. Some parameters remain unchanged before and after stress.

Table 10. *Graphical presentation of the extracted data from the videos from tank 113.*

Tank 113	Results for Data from 19-05-2023, 24-05-2023 and 27-05-2023	03-06-2023, 08-06-2023 and 11-06-2023	18-06-2023, 23-06-2023 and 26-06-2025
Area			

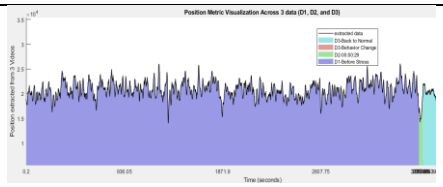
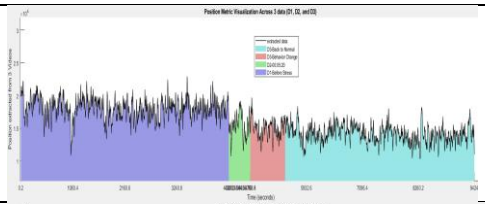
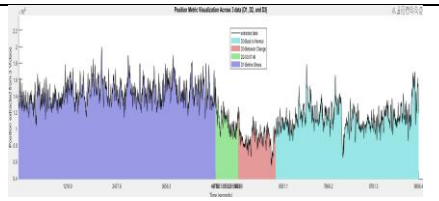
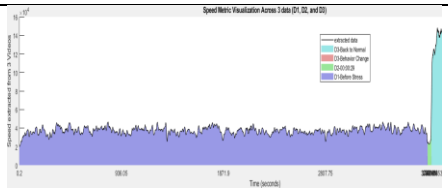
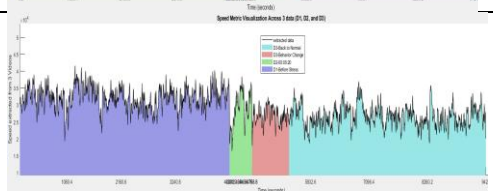
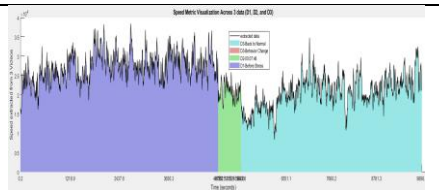
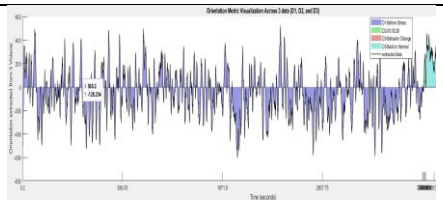
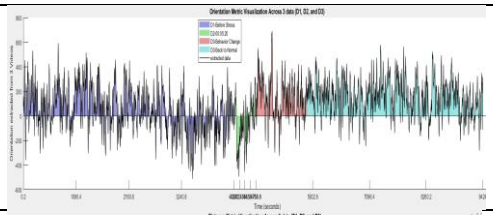
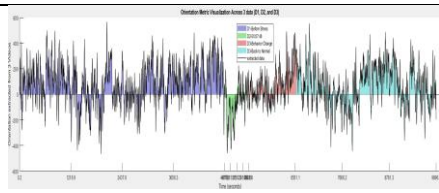
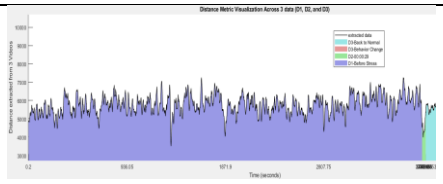
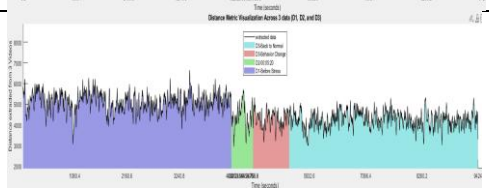
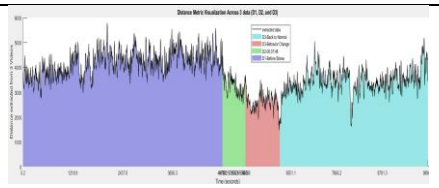
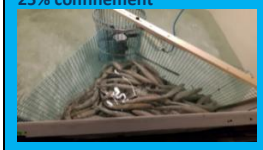
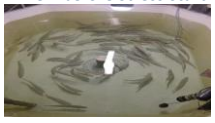
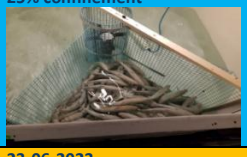
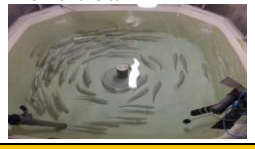
Position			
Speed			
Orientation			
Distance			

Table 11. *NOFIMA tank 115 details*

Data Before Stress(Video 1)		Data after stress recovery(Video 2)			Data 2 days after stress recovery (Video 3)	
Date	Remark	Date	Remark 1	Remark 2	Date and Tank View	RESULT
19-05-2023	Total Video Time 01:01:27	24-05-2023	Total video Time 00:02:18	The net was removed at 00:01:58, and we have 00:00:20 data to process.	27-05-2023 Total video Time 10:06:10	
	- View before Stress Start 	25% confinement 	-Some fish were removed during the stress phase, and water was filled after net removal until the end of data recording.	-View after Stress End 	-View in the tank 	After stress: -Slight spike in area. -Drop in distance, speed, and position. Refer to Table 4.1d(1).
05-06-2023	Total video Time 01:00:29	08-06-2023	Total video Time 00:11:59	Stressor or Net was not removed. Thus, we have 00:00:00 data to process	11-06-2023 Total video Time 01:04:50	
	-- View before Stress Start 	25% confinement 	-Some fish were removed during stress.	-Net was not removed 	-View of the tank 	- Refer to Table 4.1d(1).
18-06-2023	Total video Time 1:08:03	23-06-2023	Total video Time 00:11:59	The net was removed at Time - 00:03:10. Thus, we have 00:08:49 data to process.	26-06-2023 Total video Time 01:02:43	

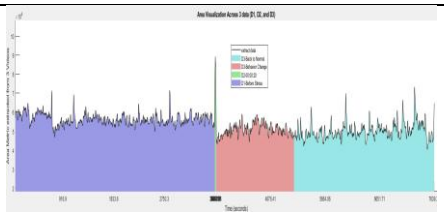
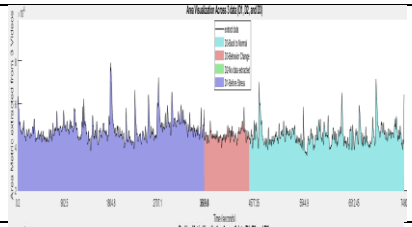
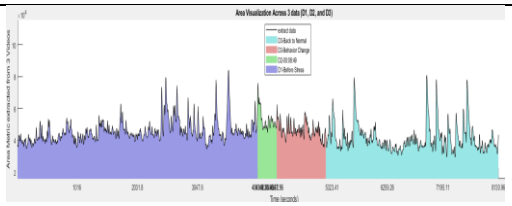
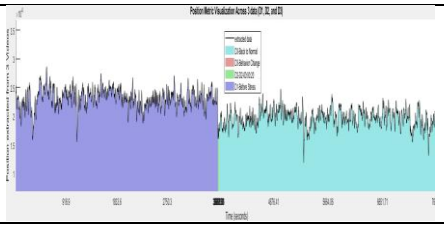
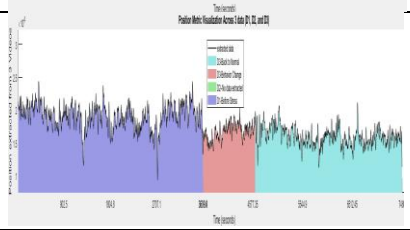
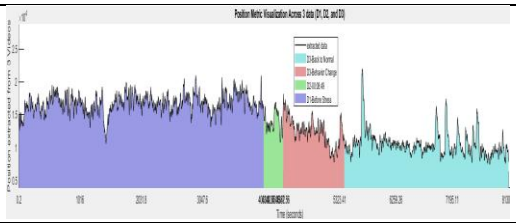
	- View before Stress Start 	25% confinement 	-Some fish were removed during the stress phase. -After net removal, unwanted movement and high-current water flow were observed until the end of the video.	-View after Stress End 	- View in the tank 	After stress: -Area appears the same -Drop in distance, speed, and position. Refer to Table 4.1d(1).

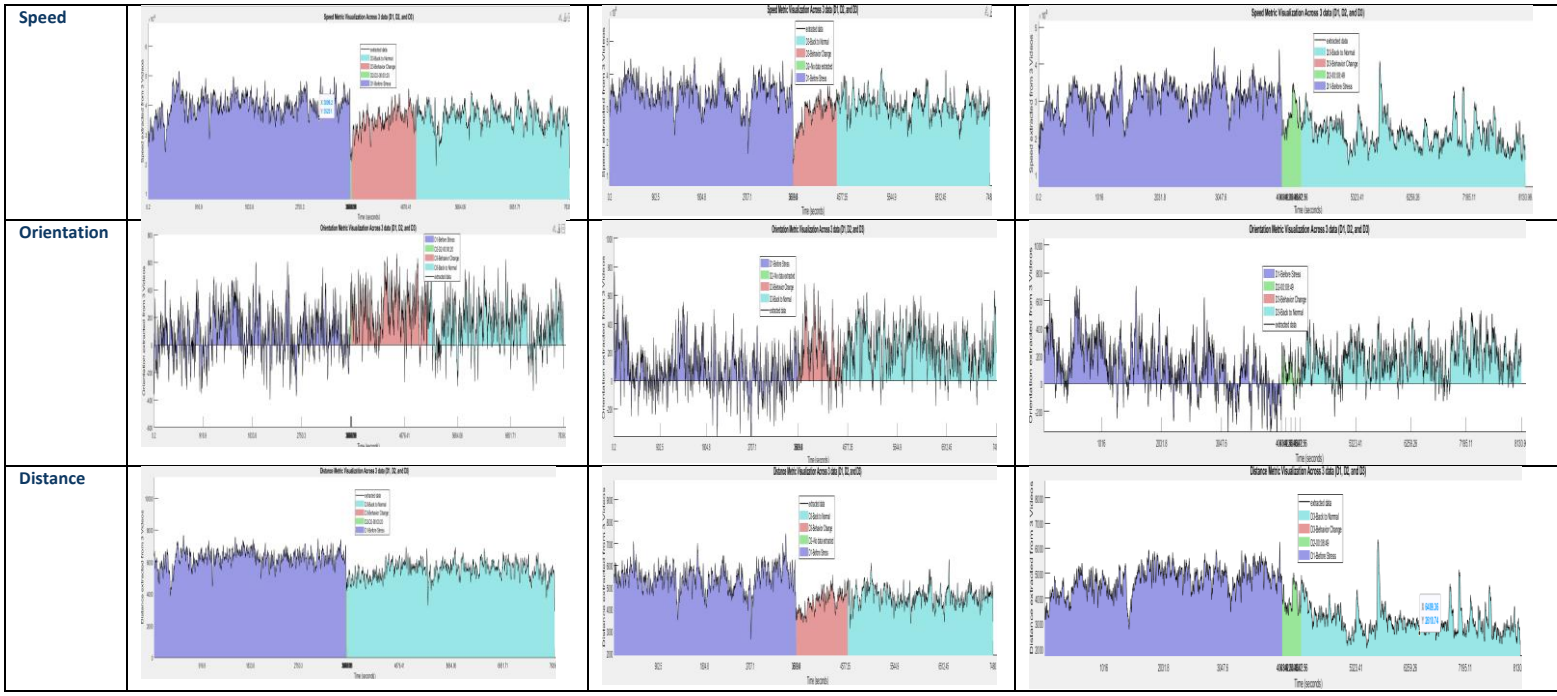
For tank 115 we have Table 12, this table documents observations from Tank 115 before stress (Video 1), data after stress recovery (Video 2), and data 2 days after stress recovery (Video 3). Here is a detailed description:

1. Data Before Stress (Video 1)
   - Video durations were recorded before stress events.
2. Data after recovery of stress (Video 2)
   - Some pictures that show fish were confined (25%) during stress using the net.
   - Fish were removed in all trials. Net removal varied—one trial had no net removal, leading to no processable data.
   - Unwanted movements and high-water currents were observed post-net removal in some cases.
3. Data 2 days after stress recovery (Video 3): Some pictures is shown on table with the video data time duration.
4. Tables 115 represent different trials, analyzing changes in fish behavior and movement.

The data metrices were extracted from these datasets Steps (1.1), results are presented into multiple sessions in Table 115, each covering different stress events and recovery periods. Observations included slight spikes in area, with drops in distance, speed, and position. In one case, no significant change in area was noticed after stress.

Table 12. *Graphical presentation of the extracted data from the videos from tank 115.*

Tank 115	Results for Data from 19-05-2023, 24-05-2023 and 27-05-2023	03-06-2023, 08-06-2023 and 11-06-2023	18-06-2023, 23-06-2023 and 26-06-2025
Area			
Position			



Results obtained by the CNN based approach

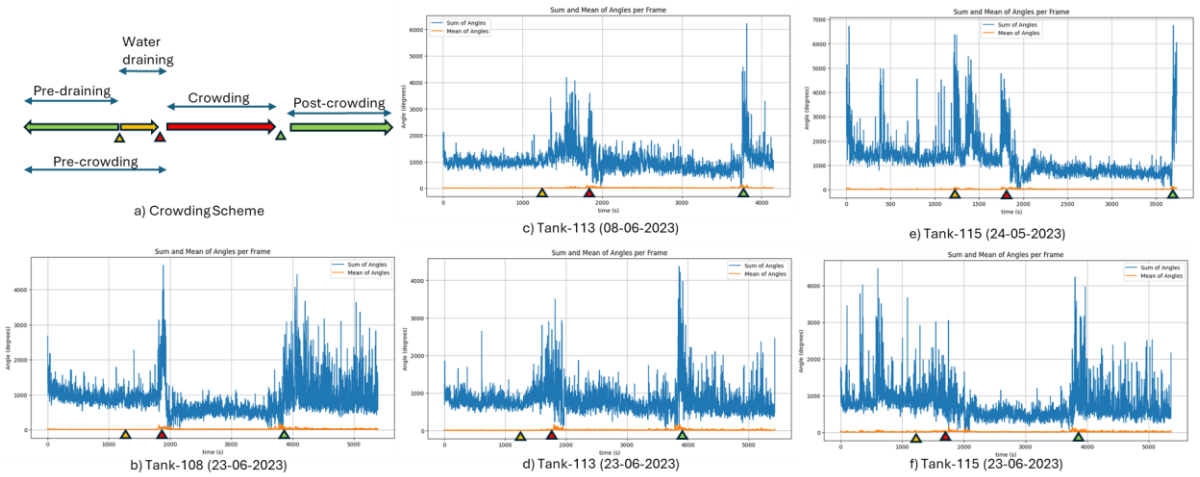


Figure 46. *Depiction of the behavioural response based on orientation score. The crowding scheme used has been shown in a) and the orientation score plotted overtime during the crowding experiments are plotted for the tanks with relevant pre and post crowding duration. Post crowding plot is missing from e) due to lack of video recording during the period.*

The results from the Nofima Behavioral Analysis Tool, as shown in Fig. 46, indicate notable differences in fish behaviour before and after crowding. During crowding, fish tend to settle down over time to normal orientation. The deviation-based orientation score effectively highlights the non-uniform distribution of fish orientation. Increased disturbance is observed in fish during pre-crowding steps, particularly during the water draining period. There is variability in fish behaviour across different tanks, influenced by external stressors such as the presence of people or random events. However, given enough time without disturbance, fish behaviour tends to normalize, effectively bringing the orientation score back to normal. Even during the crowding period, after a brief initial disturbance, the orientation score settles down, suggesting that fish adjust to crowding and orient themselves against the water current. Once crowding is over, fish initially orient themselves differently but tend to return to normal orientation over time. In summary, the tool can identify deviations from normal fish orientation without focusing on individual fish, suggesting its potential use in detecting anomalies and stress events in the tank.

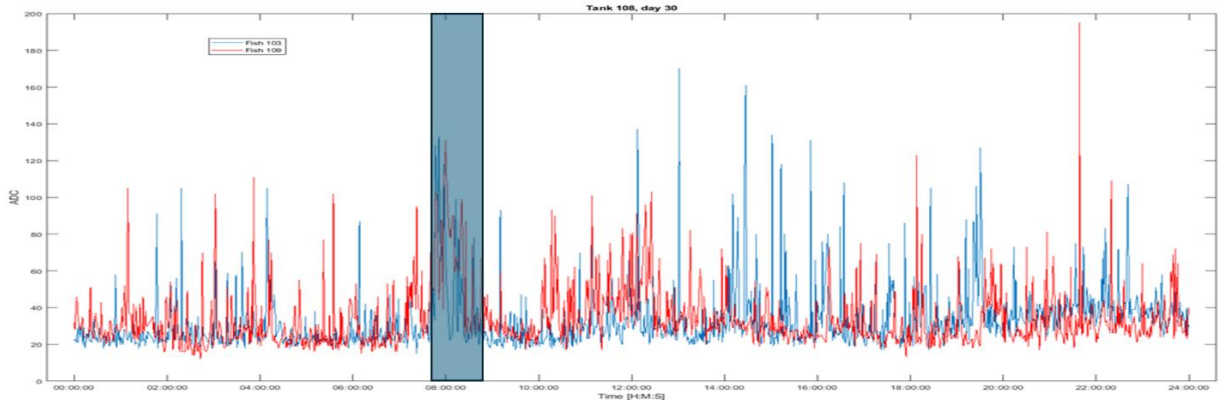
Tags results

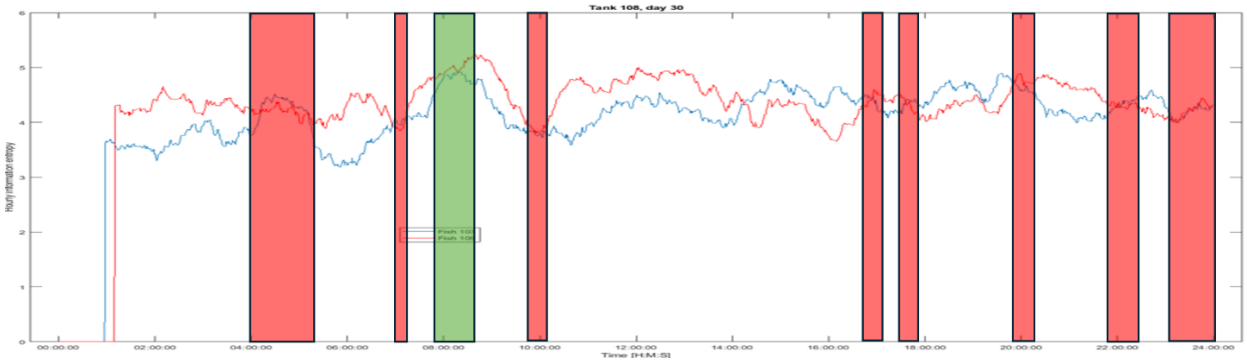
For the days of crowding experiments, individual hour distribution was evaluated by information entropy as a describing parameter of such hour, representing the amount of information given by the acceleration values distribution.

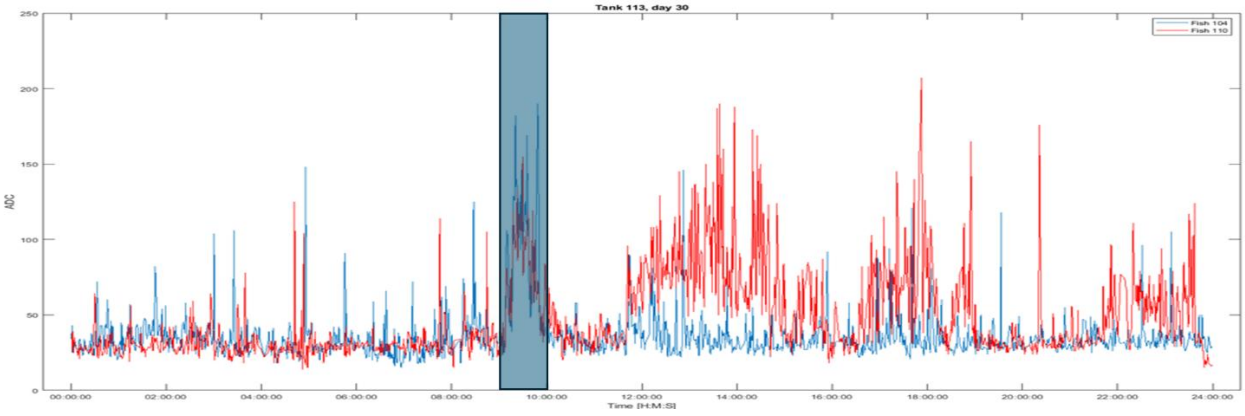
The information entropy is a variable itself, therefore also the basic statistical methods, like central moments and confidence intervals, could be applied.

Basically, it is difficult to define typical fish behaviour with exact mathematical attributes and their accepted range of values, conditionalities, and effects. However, it is possible to accept behaviour distributions of the same level of information as a behaviour, which is not surprising. On the other hand, a dramatic change in the behaviour distribution of information is a surprise. In the context of data analysis and anomaly detection, surprise refers to unexpected or atypical behaviour that deviates from the norm or expected patterns. Surprise is, therefore, always atypical behaviour.

The Fig. 47. depicts the acceleration data and corresponding entropy values data for the three tanks (108, 113, and 115) of the day when the fish cohesion was done. From the original acceleration data it is impossible to estimate the fish crowding by analysis of the signal of each fish individually. If we analyze the signals of the two fish in each aquarium simultaneously, we can detect the areas with the synchronized change of the acceleration, shown by green bars in the Fig. 46. The synchronization can be clearly seen in the values of the corresponding entropy. The entropy for both fish has almost the same value for the crowding event during a significant time span, which corresponds to the crowding period. For tank 113, the synchronization happened two times. The first one corresponds to the fish crowding, and the second (from 20:00 to 22:00) was unknown due to the missing video data.


Tank 108, day 30
Fish 103
Fish 109
ADC
Time [H:M:S]


Tank 108, day 30
Hourly information entropy
Time [H:M:S]


Tank 113, day 30
Fish 104
Fish 110
ADC
Time [H:M:S]

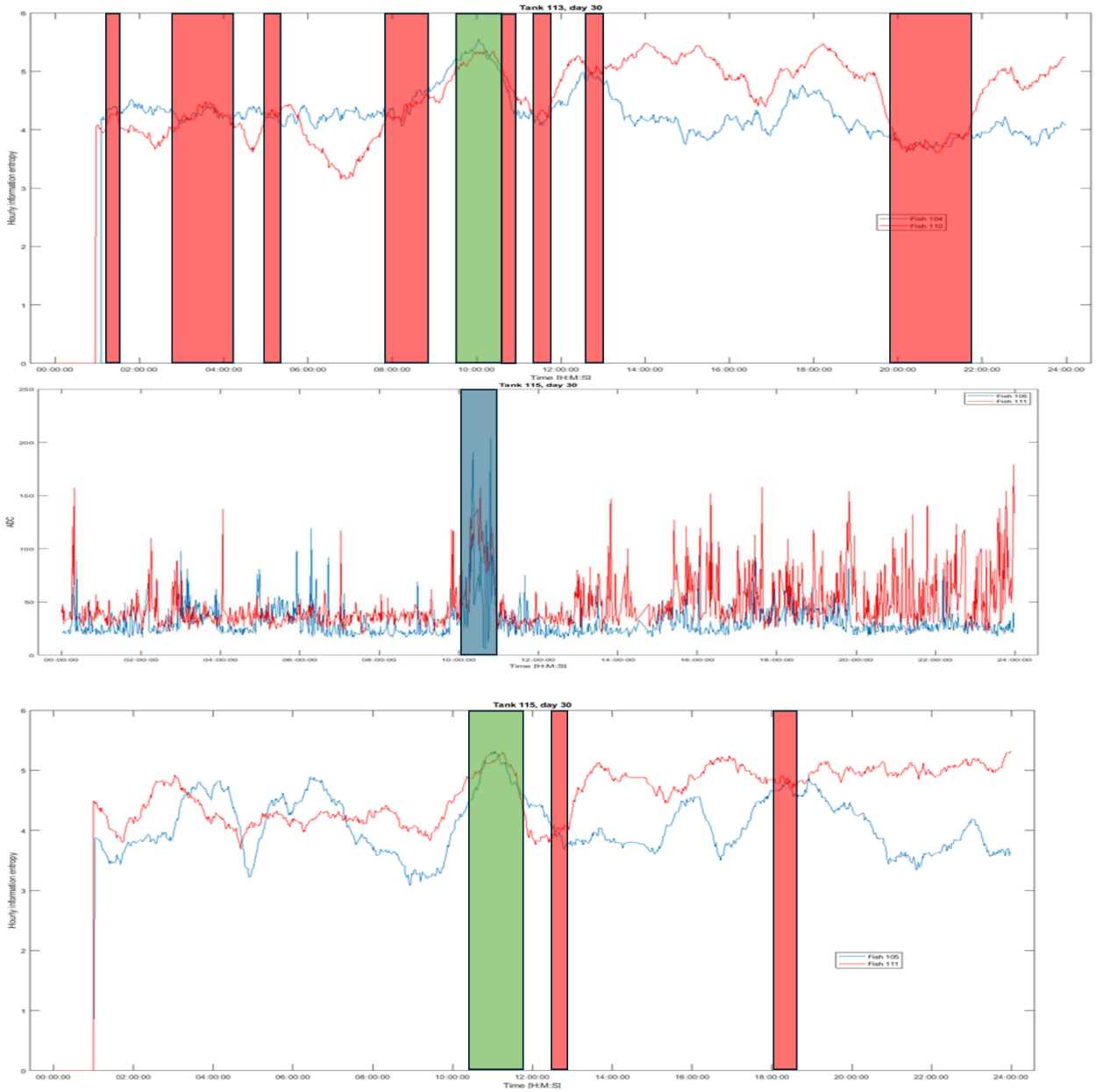


Figure 47. *Graphical view on the extracted behaviour on tank 108, 113, and 115 from telemetry and the values of corresponding entropy calculation. Blue rectangles show the fish crowding event in the acceleration data. The red rectangles depict the time intervals with synchronized changes but low level of acceleration. The green rectangles show the time intervals with synchronized changes and high value of acceleration.*

4.8. CSIC

The first trial explored the effects of high stocking density and concurrent low oxygen availability on gilthead sea bream's welfare, physiological responses, and behavioural adaptations. HD fish (25 kg/m^3) exhibited a significant reduction in feed intake and growth rates compared to LD (8.5 kg/m^3) and MD (17 kg/m^3) fish. The increased feed conversion ratio (FCR) from 1.61 in LD fish to 1.92 in HD fish pointed out reduced nutrient utilisation efficiency. According to welfare scores, HD fish showed increased external tissue damage, including scale loss and fin wear, likely due to increased contact and friction among individuals in confined space. Plasma glucose and cortisol levels were also increased with stocking density, which confirmed the stress response triggered by high stocking densities. Hepatic gene expression results (mainly down-regulation of markers of the growth hormone/insulin-like growth factor (Gh/Igf) axis, and up-regulation of genes associated with antioxidant defence [mn-sod, gpx4, prdx5] and metabolic stress responses [grp170, grp75]) suggested that HD fish prioritize oxidative stress mitigation overgrowth, likely as an adaptive strategy to cope with environmental constraints.

AEFishBIT data-loggers provided insights into behavioural adaptations under high-density conditions. As can be depicted in Fig. 48, HD fish demonstrated a stronger schooling behavior, characterized by synchronized swimming activity, and feeding time emerged as a key synchronizing factor with the acrophase of physical activity clearly surrounding the programmed feeding time in the absence of feed provision, reinforcing the social cohesion among individuals.

Over the entire diurnal recording period (09:00 to 21:00 h), correlation analyses highlighted a close positive linear correlation ($p < 0.001$) between physical activity and respiratory frequency for all tracked fish considered as a whole Figure 49(A), which in turn rendered a decreased respiration/activity ratio with the increase of stocking densities Figure 49(B). This lowered respiration-to-activity ratio would be indicative of more efficient energy utilization in HD fish. The respiration tracking rendered a strong negative correlation ($p < 0.001$) with the continuously recorded water O2 concentration Figure 49(C).

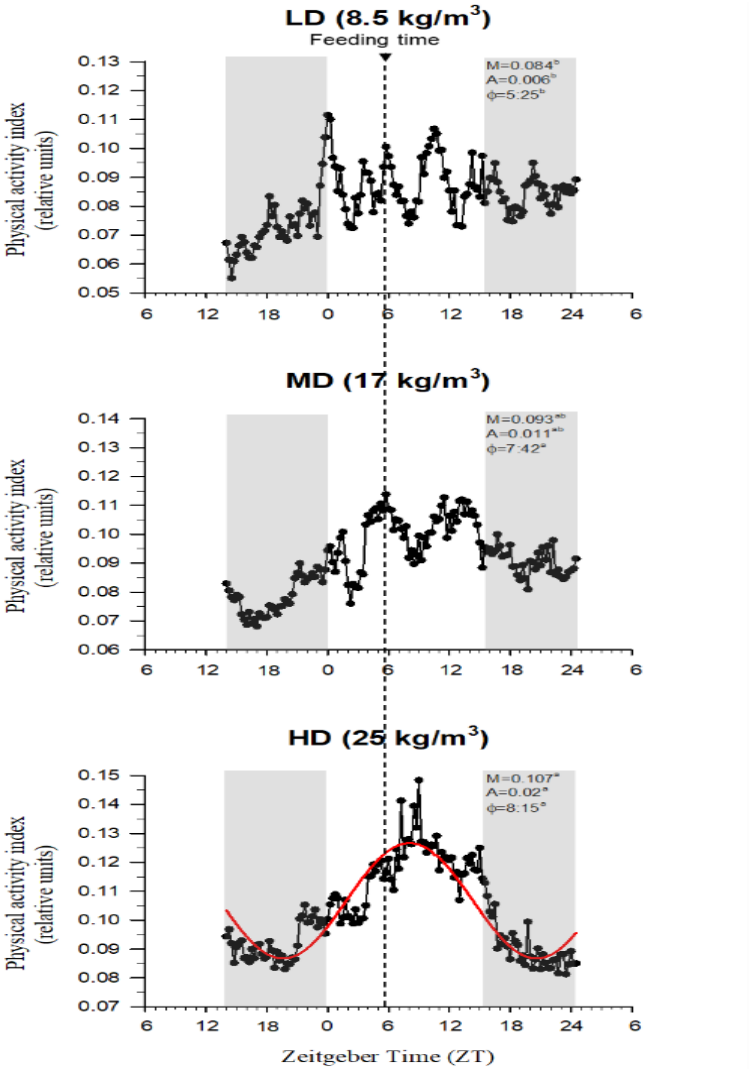


Figure 48. *Gilthead sea bream physical activity synchronization is achieved by feeding at low, medium, and high densities. AEFishBIT data (measures taken every 15 min along 2 consecutive days) of the physical activity of representative individuals (n=8) is shown as a continuous dotted line in each panel. Best-fit curve (red sinusoidal line) derived from the cosinor analysis of physical activity is only represented in the HD group. Values of mesor (M), amplitude (A), acrophase (φ) and p-value (P) of best-fit curves are shown for each density. Gray shaded areas represent dark phases. Arrow in vertical dotted line indicates the feeding time. Different letters represent significant (P < 0.05, ANOVA, Holm-Sidak test) differences between density groups.*

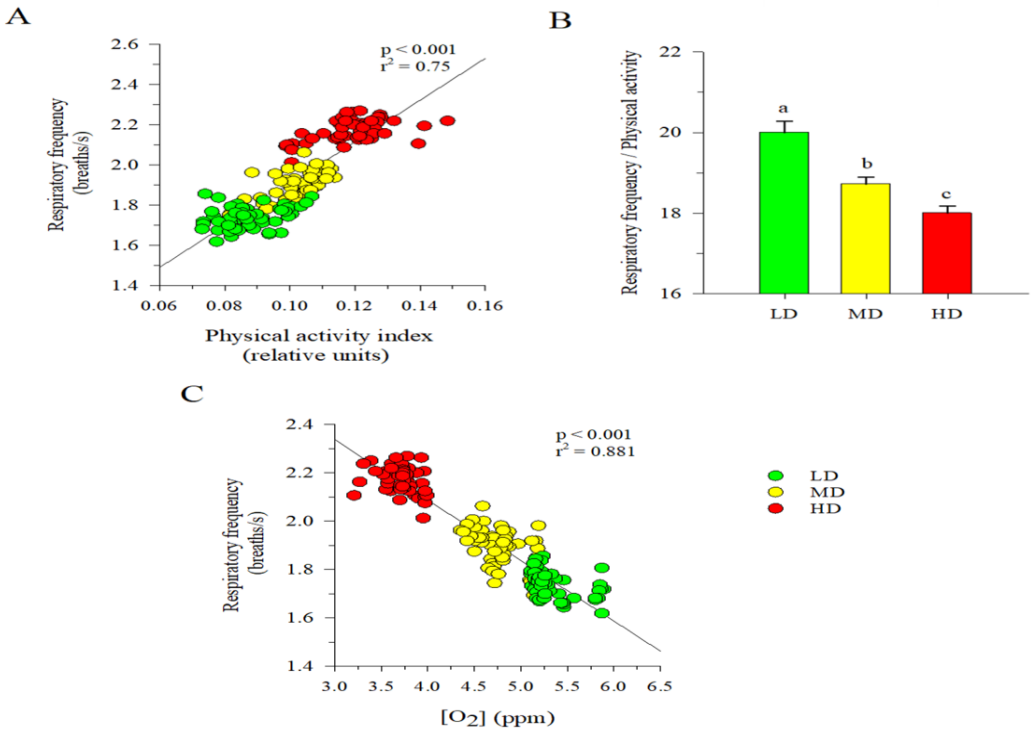


Figure 49. *(A) Correlation plot of respiratory frequency and physical activity. (B) Respiratory frequency/physical activity ratio in each stocking density group. Different letters represent significant (p < 0.05, ANOVA, Holm-Sidak test) differences between groups. (C) Correlation plot of respiratory frequency and water dissolved oxygen concentration. Values are the mean of physical activity and/or respiratory frequency (n = 8) of each density treatment and dissolved oxygen concentration each 15 min during the diurnal period.*

Trials conducted in 2022 focused on species-specific stress responsiveness and habituation to repeated confinement stress. Analysis of AEFishBIT data highlighted the different responses of gilthead sea bream and European sea bass to acute stress. Following a single confinement stressor, both species showed an immediate increase in physical activity and respiratory frequency (Figure 49). However, the magnitude of the response was species-dependent. European sea bass exhibited a more pronounced increase in physical activity compared to gilthead sea bream (Fig. 50(A)(D)), whereas the rise in respiratory frequency was similar in both species (Fig. 50(B)(E)). Recovery time for baseline activity was longer in European sea bass (3h) compared to gilthead sea bream (2 h; Fig. 50(C)(F)), indicating a higher stress sensitivity in sea bass.

To assess habituation to stress, gilthead sea bream at different stocking densities were subjected to repeated confinement stress tests. The higher stocking density induced physiological changes, such as decreased hemoglobin concentration and reduced hematocrit levels, along with slight external damage including scale shedding and minor fin wear. Muscle gene expression analysis revealed an

upregulation of myogenic regulatory factors, suggesting an enhanced muscle regeneration process rather than net muscle accretion. AEFishBIT recorded gilthead sea bream's activity and respiratory patterns before, during, and after stress exposure (Figure 51). Fish displayed an immediate response to the application of the stress test in terms of physical activity and respiratory frequency across both density groups Fig. 51(A)(B)(D)(E). The peak of the response in physical activity and respiratory frequency exhibited a smaller increase in the CTRL group compared to the HD group during both stressor exposures. Fish at high density displayed a more gradual return to baseline activity and respiration levels, suggesting a potential adaptation to chronic stress Figure 51(C)(F). Recovery time after the first stressor exposure was significantly longer than after the second exposure, indicating habituation. Fish under HD conditions exhibited less pronounced behavioural responses over time, which may suggest a higher stress tolerance.

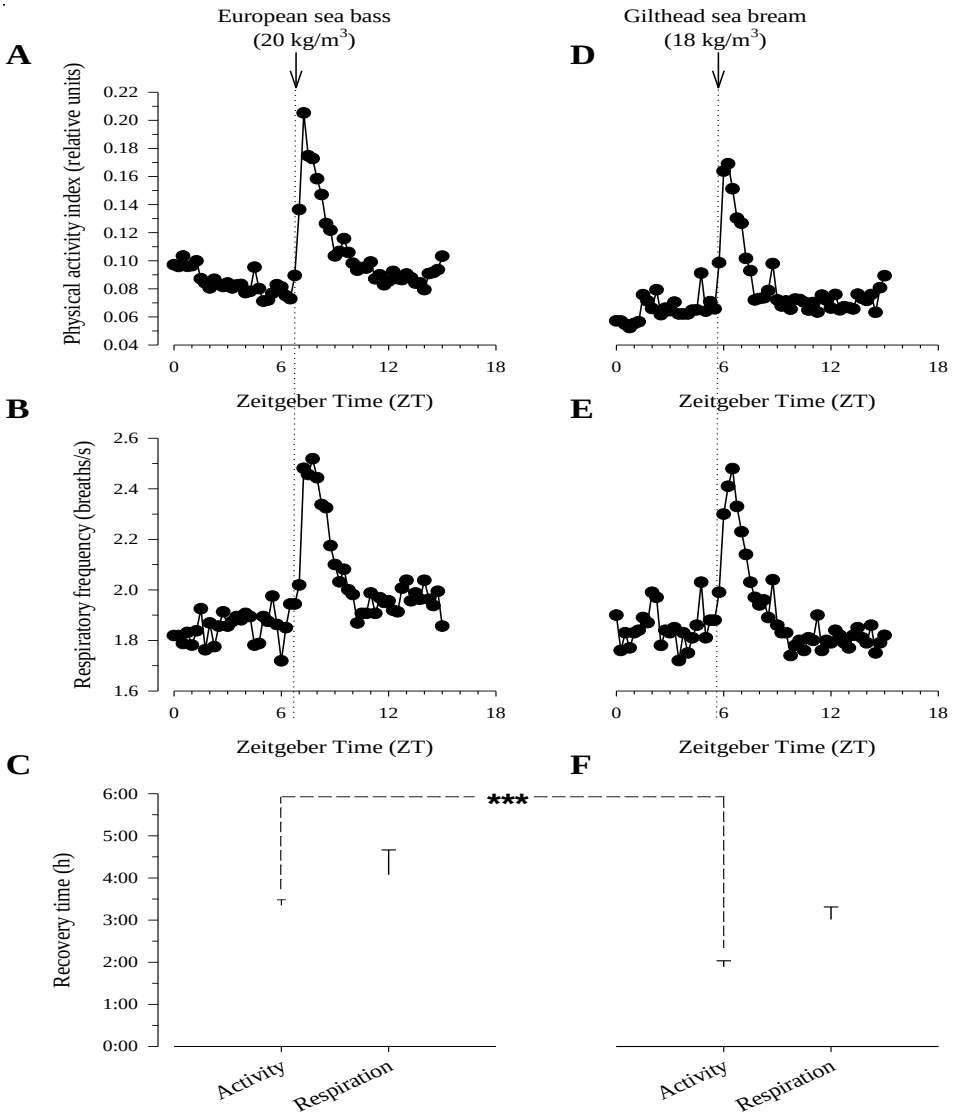


Figure 50. *European sea bass and gilthead sea bream AEFishBIT records of physical activity (A,D) and respiratory frequency (B,E) before, during, and after the confinement test. Recovery time after the stress test of both species (C,F). Measures (black circles) were taken every 15 min. Arrows in vertical dotted lines indicate the beginning of the confinement test. *** indicates significant differences between the fish species in recovery time ($p < 0.001$). Recovery time was calculated as the required time for activity and respiration to reach values close (<15% difference) to the basal levels found before the stress test of each fish (n = 8).*

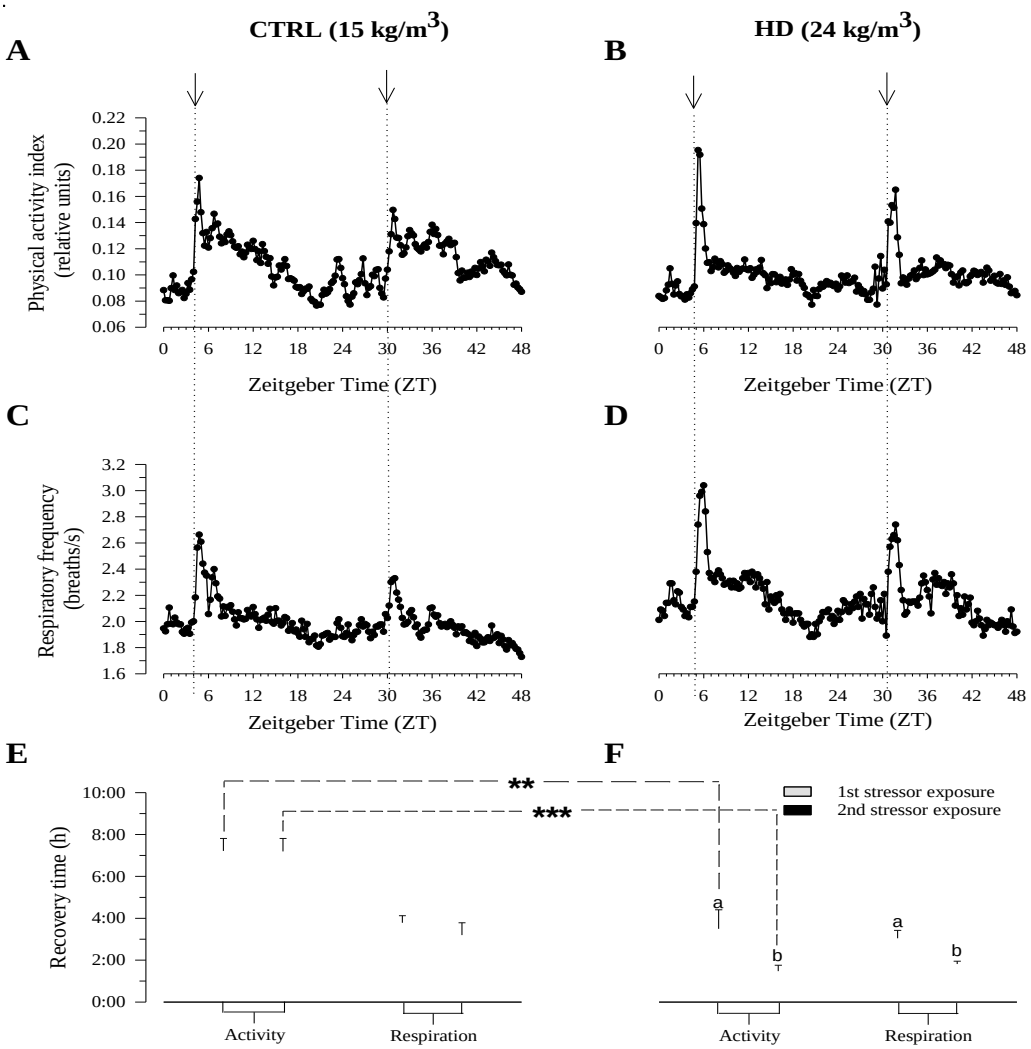


Figure 51. *Representation of physical activity (A, D) and respiratory frequency (B, E) AEFishBIT records before, during and after the confinement test in gilthead sea bream reared at two different stocking densities (CTRL 15 kg/m3; HD, 24 kg/m3). Recovery time after each stress test of both density groups (C, F). Measures (black circles) were taken every 15 min. Arrows in vertical dot-ted lines indicate the beginning of each confinement test. Asterisks indicate statistically significant differences between experimental groups in recovery time (**p < 0.01, ***p < 0.001). Different letters indicate significant differences between first and second stressor exposure (p < 0.05). Recovery time was calculated as the required time for activity and respiration to reach values close (<15% difference) to the basal levels found before the stress test of each fish (n = 7-10).*

5. Discussion

5.1. SINTEF

Video

No significant changes in swimming behaviour were measured during or after confinement. This suggests that there was no difference in behaviour, or the method was not sensitive enough to pick up subtle changes. A reason for the former could be that the confinement was not severe enough to trigger a stress response from the salmon. As illustrated in Figure 33 and Figure 34, neither average swimming velocity, swimming pattern, nor schooling behaviour showed notable shifts across the trials, with only a slight (but not significant) reduction in swimming pattern during the first confinement (Figure 32). This lack of response could indicate that the confinement duration or conditions were insufficient to disrupt the fish's normal behaviour.

Sensitivity of the method could have been influenced by the number of average fish tracked (generally <10) which may not have been representative of the entire population of approx. 190 000 fish in the cage, especially since the cameras field of view cowered a small part of the pen. Another shortcoming complicating the analysis was the lumpsucker covering the lens on April 18th. The resulting lack of data for that day Figure 32, limited our ability to fully assess the transition into the first confinement, thus impacting the sensitivity of the method.

Nevertheless, the method was able to identify the impacts of wind speed on swimming behaviour, indicating that it was indeed able to detect changes in swimming patterns, as seen in the pre-confinement period (e.g., April 13th–16th, Figure 30). The time series plot (Figure 28) shows that changes in swimming patterns and schooling behaviour align with fluctuations in wind speed, particularly during high-wind events.

However, this correlation might not solely reflect the fish's response to wind; it could also be due to an increase in camera movement during high waves, potentially affecting tracking accuracy or influencing the fish indirectly through altered visuals. This gap highlights a vulnerability in the data collection process that needs addressing in future studies.

Echosounder discussion

The echosounders observed an increase in fish density in the upper meters of the cage during crowding. This can be considered an obvious reaction to the reduced availability of space after shortening the net's side walls and effectively reducing the cage volume.

While fish distribution was typically quite even throughout the cage prior to crowding, some deviations from this pattern were observed where fish rapidly moved away from one side of the net wall or favoured the sides over the middle. During crowding, there was less variability in fish distribution. A potential reason for this may be the reduced availability of space, encouraging the fish

to use the remaining cage volume. However, also during crowding, similar movements of fish to the cage side were observed to a smaller extent. Possible reasons for the fish 'shying away' from one side of the cage may be caused by boat activity or something similar, often noise-emitting operations concentrating on one side of the pen or a neighbouring pen.
This may be further supported by observations made during net cleaning of the monitored pen. Fish showed clear movements towards one cage side during net cleaning, likely caused by the movement, noise and particle emissions coming from the cleaning robot operated inside the pen.

The echosounders, as such, work well in monitoring the fish and their position in the cage. However, the crowding carried out in this experiment was not intense enough to provoke any significant changes in behaviour. In comparison, net cleaning, which was carried out both on the monitored cage and also nearby cages, appears to have affected fish behaviour more than the crowding itself, resulting in a more distinct reaction. For future experiments, it could be worthwhile to inspect AIS-data recording ship movements and thus potential disturbance of the cage environment or get a more detailed log from the site to determine potential causes for sudden behavioural changes.

5.2. CSIC

The Video recorded data from CSIC provides valuable insights that align well with our desired parameters for analyzing fish behaviour. Observations from the video clearly indicate that during 33% confinement using the net and after its removal, the fish exhibited noticeable signs of stress, particularly in their movement patterns, as perceived by the human eye. This visual assessment is further supported by the extracted parameters, which reveal statistical variations in speed, distance, area, and position, confirming that the fish underwent behavioural changes in response to stress. Moreover, the data suggests that after the removal of the net, the fish took some time to return to their normal movement patterns, highlighting a delayed recovery phase. These findings reinforce the effectiveness of our proposed method in capturing behavioural changes through measurable parameters. However, as depicted in Fig. 45 , the orientation parameter does not provide substantial insights into fish behaviour, suggesting that it may not be a critical factor for assessing stress responses in this context.

AEFishBIT has proven to be an effective tool for monitoring behavioural responses in farmed fish. Results from the first study on gilthead sea bream suggest that high stocking densities drive behavioural synchronization and energy-saving strategies in gilthead sea bream. This behavioural adjustment suggests that high stocking densities promote proactive behaviour strategies, allowing fish to minimize energy expenditure while maintaining group cohesion. The observed reduction in the respiration/activity ratio in HD fish may indicate a physiological adjustment to cope with lower oxygen

availability, prioritizing maintenance over growth. Additionally, correlation between increased activity synchronization and local muscle growth regulation suggests an adaptive mechanism supporting proactive rather than reactive behaviour. However, despite these adaptations, the trade-off between reduced growth rates and increased external damage highlights potential welfare concerns. These findings underscore the importance of optimizing stocking densities to balance production efficiency with fish welfare, as well as the importance of monitoring tools such as for precision aquaculture management.

AEFishBIT data also served to highlight species-specific differences in stress sensitivity and coping mechanisms. European sea bass, a predatory species, displayed a more intense and prolonged reaction to stress, whereas gilthead sea bream recovered more quickly. This suggests that gilthead sea bream may be better suited to high-density aquaculture conditions, as their natural schooling behaviour facilitates adaptation to crowding. Additionally, the faster habituation observed in HD-reared sea bream suggests that prolonged exposure to high stocking densities may promote behavioural plasticity, reducing the physiological cost of stress over time. Measures of behavioural habituation will contribute to disclose the farmed fish habituation to a given environmental condition, contributing to refine the tolerance range of a particular set of stressors.

5.3.HCMR

Our results indicate that stressor events can modify fish behaviour in non-trivial ways. The type of stressor applied to the fish and its intensity plays a role in the expected response. For example, net cleaning and a significant decrease in the volume clearly affected the fish behaviour, but only for the first time. It seems that when fish are subjected to repeated stress in a short period of time, they show a weaker behavioural response. This is probably due to the fact that the stressors applied in the study and the duration of the experiment were not detrimental to the fish to show a more permanent deviation from normal behaviour. Moreover, the convergence of swimming speed between treated and control fish, as seen in the E. seabass tank experiment, indicates early signs of habituation to the repeated stressor for the treated fish.

This study used a single-vision camera in two different setups, the open sea cage and the RAS. We showed that the technology used here can detect differences in behaviour even after applying weak stressors for a short period. However, each system has challenges when it comes to the collection of data, something that can lead to the acquisition of noisy data. In the case of open cages, the problem lies in the extremely variable environmental conditions that can affect the daily accuracy of the camera recording, as well as the high densities of the fish that can create difficulties in acquiring long trajectories. In addition, turbidity and variable lighting conditions can lower the data quality. All these

can result in high variability around the actual values, something that we see in the data presented here. Smoothing and filtering methodologies are very useful tools to eliminate noise, and they have been used in the current study. Constant improvement of the technology and the data analysis tools can continuously improve the accuracy of the results and increase confidence.

In the case of RAS systems, the conditions are a lot more controlled and stable. However, problems of high density and the shape of the tanks can affect the data acquisition process and the analysis. The RAS consists of cylindrical tanks of high depth relative to the perimeter of our facilities. In addition, the background is dark, and the fish do not have high contrast when they are at the bottom. Reflection problems can also occur from the lighting. All the above can challenge accurate data acquisition, and noise is also unavoidable. For this reason, different image analysis techniques can be applied to take advantage of the peculiarity of each system. For example, the analysis of the surface preference was used because of the particularities of the current RAS system, i.e. the low contrast found at the bottom. This methodology may not be useful as such in different aquaculture setups (either in RAS or in cages). The reflection of the light was minimized by carefully selecting the position and orientation of the lights above the tank. Regarding the analysis, correctly selecting the methodologies for filtering and data analysis is very important. In the RAS experiment, the data for the acute response analysis were noisy, hindering the trends around the stress events. By fitting a polynomial curve, we could easily analyze the data and detect change points with consistency.

Sources of noise that need to be addressed in the future for better analysis include the minimization of lighting variation in RAS and robust methodologies for lighting homogenization in images drawn from cages. The selection of appropriate tanks and background colors for improved detection is necessary for improved data acquisition. Improvement of the AI models and Computer Vision techniques to address occlusions and moving backgrounds is also necessary.

5.4. NOFIMA

The Nofima Behavioral Analysis Tool has demonstrated significant utility in monitoring and analysing fish behaviour under varying conditions. The tool's ability to highlight deviations in fish orientation provides valuable insights into the impact of crowding and other stressors on fish behaviour. Notably, the tool effectively captures the differences in behaviour before and after crowding, with fish tending to settle down over time during crowding periods. This settling behaviour is reflected in the orientation score, which normalizes given sufficient time without disturbance.
The increased disturbance is observed during pre-crowding steps, particularly during the water-draining period. This is crucial for understanding the stress responses of fish. Also, the variability in behaviour across different tanks, influenced by external factors such as the presence of people or

random events, underscores the importance of considering environmental stressors in behavioural studies.

The tool's ability to detect anomalies and potential stress events without focusing on fish group suggests its robustness in identifying overall trends and deviations in fish behaviour. This feature is particularly useful for large-scale monitoring and ensuring the well-being of fish in aquaculture settings. The normalization of behaviour post-crowding further supports the tool's effectiveness in tracking recovery and adaptation processes.

However, there are limitations to this methodology. As the analysis relies on fish orientation, it is crucial that recordings are obtained from top-mounted cameras to ensure accurate data collection. Issues such as reflections and turbidity in the water can hinder detection, potentially impacting the interpretation of the deviation score. These factors must be carefully managed to maintain the reliability of the tool's assessments.

The Tool proves to be highly valuable in behavioural studies, offering valuable insights into fish responses to crowding and environmental stressors. Its application can enhance the management and welfare of fish in aquaculture research settings, contributing to more sustainable and efficient practices given relevant recording can be obtained.

Classical image processing

On the other hand, the data from NOFIMA using the Step 1, for data collected from tanks 108, 113, and 115, proved to be much more challenging to analyze. The recorded data was significantly affected by disturbances, primarily due to excessive movement caused by the water supply force in the tank, the removal of fish during the stress phase, and other unwanted movements or disturbances, as depicted in Table 7, 9 and 11. These factors introduced inconsistencies in the dataset, making it difficult to establish a clear behavioural pattern.

Additionally, the removal of fish during the stress phase was not ideal for this observation. Since the video data before stress had a higher number of fish in the tank and the video data after stress had a lower number of fish, the inconsistencies in population further complicated the analysis. The variation in fish count made it challenging to draw reliable conclusions, as the differences in movement patterns could be attributed to the reduced number of fish rather than actual stress-induced behavioural changes.

Moreover, the sequence of recorded data, particularly after stress application or net removal, contained insufficient data, making it extremely difficult to compute metrics or extract meaningful insights. The short duration of the recorded videos further limited the ability to analyze plotted graphs and observe behavioural changes. Another major challenge was the availability of post-stress recovery data recorded two days after the stress event. This delayed recording made it even more difficult to determine whether any behavioural changes occurred in response to stress, as the fish may have already adapted or returned to normal behaviour by the time data was collected.

5.5. Summary discussion

As demonstrated by the presented results, various technologies exhibited differing levels of effectiveness in detecting short-term stress events that simulated changes in fish welfare conditions. The harmonization of the experimental design led to the selection of relatively simple stressors applicable across all fish species and rearing unit types. However, not all experiments fully adhered to the experimental protocol, and most faced challenges that should be addressed in future studies employing these advanced technologies.

The overarching conclusion from all studies indicates that camera systems, echo sounders, and activity-monitoring tags are capable of detecting behavioural changes in fish, both in cage and tank environments. Behavioural alterations were observed across all three fish species and all experimental infrastructures. These changes were consistently associated with stress induced by reduced swimming space, except in the case of Atlantic salmon in sea cages in Norway, where such behavioural changes were not detected. Nevertheless, the analysis of camera footage in this setting revealed the ability to detect changes in fish swimming speed in response to variations in wind speed, while echo sounder data processing demonstrated the capability to identify spatial preferences within the cage, specifically favouring areas not subjected to net cleaning. Net cleaning itself can be regarded as a potential stressor, although the stress responses of fish to this stimulus remain insufficiently investigated.

Each technology applied in this study, along with its corresponding data processing methods, possesses distinct advantages and limitations. The echo sounder, used for monitoring salmon in sea cages, proved highly effective for assessing the overall distribution of fish within the rearing unit and tracking the dynamics of spatial changes. However, its limitations include the inability to monitor individual fish behaviour and its unsuitability for tank environments. From the perspectives of maintenance and data processing, the echo sounder emerges as the simplest technology.

The constraint of not capturing individual behaviours is mitigated by the use of acceleration tags and video cameras. Acceleration tags successfully detected stressors in fish held in tanks. The primary advantage of this technology lies in its lower sensitivity to environmental factors, particularly water turbidity. Additionally, data processing from these sensors is less complex than video data analysis. Nonetheless, a significant drawback is the invasive nature of sensor attachment to or insertion into the fish's body. AEfishBIT tags, though externally attached, do not support real-time data transmission and are therefore suitable for post hoc welfare assessment over specified time periods. Retrieval of data requires physical capture of the fish. Conversely, the acoustic acceleration tags used in the NOFIMA experiment enable real-time data transmission but are considerably expensive, which limited

their deployment to only two fish per tank. While acceleration tags offer highly precise data, their invasive application remains a clear disadvantage.

The final technology assessed in this study was the video camera system. A prominent issue with this technology is the dependence of video quality on water clarity, and the potential obstruction of the camera's field of view by objects in the environment. Furthermore, in sea cages, the area monitored by a single camera is limited. A significant technical challenge was the mounting of cameras within the sea cages, as water currents could cause camera displacement, thereby distorting the perceived movement of the fish. However, video technology provides the clear advantage of enabling the observation of both individual fish behaviour and group dynamics. Image analysis techniques allow for the examination of short-term behavioural characteristics, from which numerous behavioural parameters can be extracted. This is simultaneously a strength and a limitation of video technology, as data processing often requires substantial computational power and complex analytical methods. In this study, conventional image processing techniques were compared with advanced methods based on convolutional neural networks (CNNs). Both approaches demonstrated the capacity to extract behavioural descriptors; however, CNN-based methods require annotated training data, whereas conventional methods necessitate the adjustment of processing parameters to suit specific data quality conditions.

Certain limitations in experimental execution partially affected the conclusiveness of the findings. The experiment conducted by CSIC with gilthead seabream yielded a sufficient dataset from AEfishBIT sensors but included only a single video recording of the spatial restriction stressor. Consequently, it remains unclear whether the behavioural changes observed in the video recording are reproducible. Comparison between the video analysis results and AEfishBIT data suggested a temporal shift in the fish's response to confinement. This discrepancy may be attributable to the inherent differences between the two data sources and the averaging process used for AEfishBIT data. Nevertheless, a clear correlation was observed between behavioural changes identified through video analysis and those indicated by the tag-derived activity data.

In the experiments conducted by NOFIMA, video footage of salmon following the confinement stressor was captured two days after the event. By this time, the fish had already recovered from the stressful situation, as confirmed by continuous acceleration data monitoring. However, the detection of the stressor was achieved using several very short video recordings taken immediately after confinement. These recordings clearly indicated behavioural changes, although repeating the experiment would be advisable to validate the observations.

The experiments demonstrated that, despite the harmonization of experimental conditions and the stressor applied, changes in fish behaviour are highly species-dependent. Stress detection in sea bass

within the tank environment was straightforward. Following the application of the stressor, the fish exhibited a clear preference for the bottom of the tank, and the simple detection of fish presence near the water surface correlated well with the occurrence of stress. Furthermore, the experiments revealed fish adaptation to repeated exposure to the stressor, as subsequent applications no longer elicited such pronounced behavioural changes. In the case of stress detection in salmon, it became evident that behavioural responses in tanks differed markedly from those in sea cages. This discrepancy is influenced by several factors, underscoring the necessity for data collection and analysis methods to be specifically tailored to tank environments and sea cages, respectively.

Through comprehensive analysis, it was demonstrated that behavioural parameters extracted using different technologies exhibit strong correlations. Video recordings from cage environments provided comparable information regarding fish density to that obtained from echo sounders. Similarly, acceleration tags detected activity trends consistent with those identified via video cameras. Accordingly, a multi-technology approach is recommended for effective welfare monitoring. Each technology offers specific benefits, and cross-validation of results from multiple systems can aid in identifying potential errors in data analysis or sensor measurement.

6. Conclusion

In conclusion, the multi-tech approach of this study demonstrates that different monitoring technologies are complementary in assessing fish stress. Video and image analysis provide direct behavioral observation, echosounders offer reliable whole-cage occupancy data, and biosensors yield in-depth physiological metrics. Each is best suited to particular scenarios – for instance, acoustic monitoring in large sea cages and visual/movement tracking in small clear-water systems – and each faces unique hurdles. To achieve real-time, farm-wide stress detection, future development should focus on integrating these technologies into a cohesive system: one that can leverage the strengths of each method while compensating for their individual limitations. By addressing the current drawbacks (sensitivity to mild stress, environmental noise, habituation effects, and deployment practicalities), such an integrated monitoring framework could greatly enhance our ability to detect signs of stress in aquaculture, leading to more responsive farm management and better fish welfare in the long run. The experiment and analysis documented in this study demonstrated the possibility of detecting short-term stressors for several species, but to be able to generalize to more stressors causing the change of the welfare, more experiments must be performed.

7. References

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8. Glossary/Definitions

AQUAEXCEL3.0: AQUAculture Infrastructures for EXCELlence in European Fish Research

9. Appendixes

- **Experimental design**

Document Information

EU Project	No 871108	Acronym	AQUAEXCEL3.0
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Deliverable	N°	6.4	Title	General determination of welfare indicators across the fish species using the monitoring technologies in tanks and cages
Work Package	N°	6	Title	Welfare indicators
Work Package Leader	Chris Noble			
Work Participants				

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