# Using behaviour of herring (*Clupea harengus* L.) to assess post-crowding stress in purse-seine fisheries

Thesis in partial fulfilment of the degree

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Rachael Morgan

Department of Biology

University of Bergen

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# Abstract

Within the purse-seine fishery crowding fish at high densities, during the process of slipping, has been shown to cause high mortalities. Mitigation measures are therefore needed to enable fishermen to avoid slipping fish, particularly when they have a low survival likelihood. Providing a means of assessing the welfare of the catch may provide a solution and behaviour has the potential to be used as an immediate welfare indicator.

The behavioural effects of crowding Atlantic herring (*Clupea harengus*) in purse-seines were analysed from video-footage collected in large-scale field survival experiments. Crowding densities ranging from 1.5-351 kg/m$^3$ were simulated to represent the final stages of purse seining and the process of slipping.

A detailed and systematic analysis of several behavioural metrics, describing swimming activity and orientation, is described, including tail beat frequency and amplitude, swimming mode, vertical and horizontal orientation and nearest neighbour distance.

Tail beat frequency increased with crowding density and was strongly correlated with mortality. This, combined with a decrease in tail beat frequency from the start to the end of the experiment, suggests that high crowding densities may cause fish to swim to exhaustion. Swimming patterns within each cage were diverse and tail beat amplitude was also exceptionally high compared to other studies. Captivity effects may have impacted some aspects of herring behaviour, as orientation showed unexpectedly large variance across all crowding densities and poor alignment, suggesting a lack of schooling. The occurrence of extreme orientations increased over the duration of the experiment, possibly due to increasing sea bird predation. Orientation did not appear to be influenced by crowding density. Tail beat frequency seems to have the best potential as an indicator of subsequent mortality.

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# 1. Introduction

The release of fish from purse-seines, commonly known as "slipping", has been shown to cause high levels of mortality if the fish have been heavily crowded within the net prior to release (Lockwood *et al.*, 1983; Huse and Vold, 2010; Tenningen *et al.*, 2012; Marçalo *et al.*, 2006). This unaccounted mortality raises concerns about fish welfare and may lead to biases in stock assessment (ICES, 2004; 1997).

Globally purse seines are the most productive fisheries (Watson *et al.*, 2006) and in Norway accounted for 610 718 tonnes of Northeast Atlantic herring (*Clupea harengus* L.) in 2012 (Fiskeridirektoratet, 2013). Purse seines are designed for catching schooling fish. Once a target school has been identified, the purse seine is shot and the vessel is used to surround the school with the net. The bottom of the net is drawn up, to close the net, forming the purse (Lockwood *et al.*, 1983). The net is then heaved on-board the vessel and the catch is concentrated in the bunt end of the net. Herring catches may weigh over 1000 tonnes and the fillet price, per kg, can double depending on the quality of the fish (around 3-6 NOK per kg; Norges-Sildesalgslag *pers. comm.*). Fishermen therefore have a strong incentive to maximise their profits by catching the highest quality fish. It is difficult to determine the size and quality of the catch before the fish have been densely crowded in the bunt of the net, at the end of the haul, with densities exceeding 250kg/m$^3$ (Tenningen *et al.*, 2012). At this stage, if the quality is poor, the size of the fish too small or the catch simply too large for the vessels capacity, the catch is considered unsuitable for market and will be released or "slipped" (Breen *et al.*, 2012). In Portugal the main reason for slipping sardine catches is daily quota limitations (Stratoudakis and Marçalo, 2002); filling an annual species quota is also a reason for slipping in Norway.

The term "slipping" differs from discarding as it refers to fish being released from the net without being hauled onboard (Stratoudakis and Marçalo, 2002). As the fish remain in the water it was assumed slipped fish survived, which was supported by fishermen's observations of the fish swimming freely out of the net. In the 1970s demersal trawls in the North Sea started to report catches of dead mackerel (*Scomber scombrus*; Lockwood *et al*., 1983) which prompted research into the effects of slipping in purse seining. Both lab and field-based experiments have shown that the high crowding densities prior to slipping may lead to high rates of unaccounted fishing mortality in herring (Tenningen *et al.*, 2012), mackerel (Huse and Vold, 2010; Lockwood *et al.*, 1983) and sardine (*Sardina pilchardus*; Marçalo *et al.*, 2006). In 2007 ICES reported that landed catches alone did not explain the significant reduction in Northeast Atlantic herring; slipping could be one explanation for the unaccounted mortality (Huse and Vold, 2010). Slipping is therefore also a concern for the accuracy of stock assessments, which do not include unaccounted mortalities (Tenningen *et al.*, 2012). The frequency of slipping catches is unknown due to difficulties in monitoring; one experiment in Portugal estimated the amount slipped as more than two thirds of the total catch (Stratoudakis and Marçalo, 2002).

The mortality of herring has been found to correlate with crowding density, with low crowding densities having a higher survival rate than high crowding densities (Tenningen *et al.*, 2012). Survival experiments for herring in 2008 and 2009 found that densities less than 150 kg/m$^3$ did not result in mortality significantly greater than the control group. However a density of 221 kg/m$^3$ showed 28% mortality and at the highest densities of 403 kg/m$^3$ mortality was as high as 52% (Tenningen *et al.*, 2012). This crowding density is likely to represent the density herring are exposed to immediately prior to slipping.  If a decision was made, whether to retain or release a catch, before increasing the crowding density to such high levels, the unaccounted mortality of the slipped fish could be decreased. Similarly in the study described in this thesis, which used data from 2012, the mortality was also shown to be correlated with crowding density with the highest mortality of 27.8% found at the highest crowding density 351 kg/m$^3$ (Figure 1).

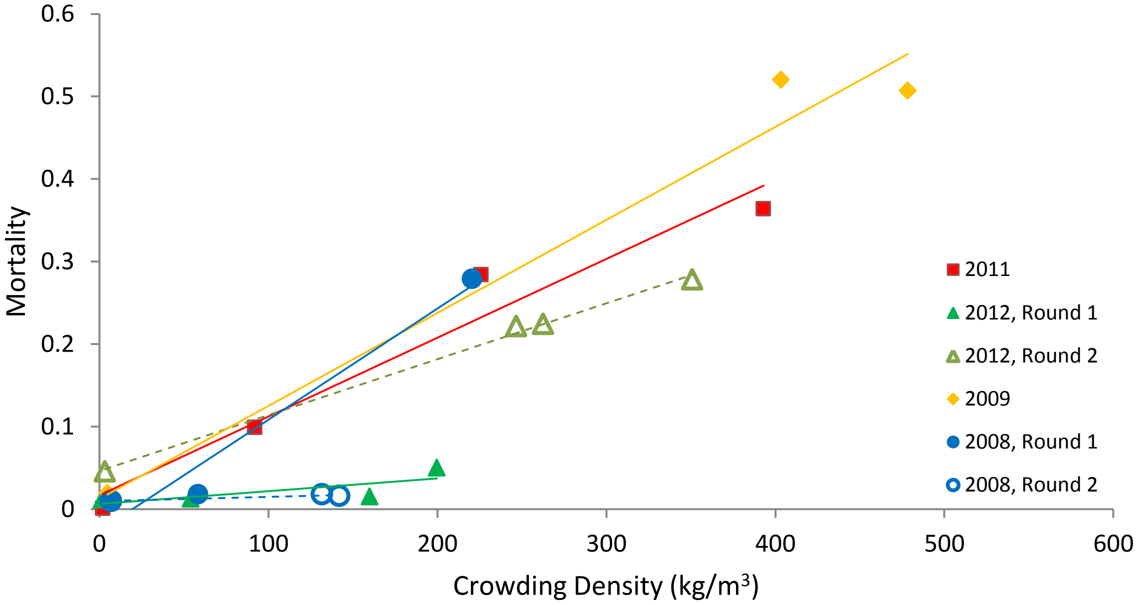


Figure 1: The relationship between crowding density (kg/m$^3$) and mortality in survival experiments conducted in 2008 & 2009 on North Sea herring and 2011 & 2012 on Norwegian spring spawning herring. The 2012 data is used in this study (Breen & Vold, *pers. comm*. adapted fromTenningen *et al.* (2012)).

In order to understand why herring have such high mortality rates when exposed to high crowding density we first need knowledge about their behaviour in the natural environment.

## 1.1 Behaviour of herring

Herring are an obligate schooling species (Partridge *et al.*, 1980) which school in all phases of their lives (Nøttestad *et al.*, 2004). Schools can be defined as groups of fish which are maintained by social interactions and which exhibit synchrony of orientation and direction of travel (Grunbaum, 1998). The main benefits of schooling are decreased risk of predation (Pitcher and Parrish, 1993) and decreased foraging time (Pitcher *et al.*, 1982); these factors influence the density, size and structure of schools (Pitcher, 1986). Herring use vision and the lateral line to orientate with their neighbours and retain the schooling structure: vision to maintain position and angle between fish; and the lateral line to determine the speed and direction of their neighbours (Nøttestad *et al.*, 2004).

Compared to other species, such as saithe (*Pollachius virens*) and cod (*Gadus morhua*), herring show a proportionally larger interfish distance, to provide more space for the herring to change direction in response to a predator, as they have stiffer bodies than saithe and cod (Partridge *et al.*, 1980). The interfish distance decreases, as school size increases (Partridge *et al.*, 1980), meaning the school becomes more dense, with herring school densities ranging from around 0.3-22.2 fish/m$^3$ (0.06-4.44 kg/m$^3$) (Misund *et al.*, 1995). Herring form extremely dynamic schools with the ability to change their structure within seconds, an adaptive feature in predator defence. A study by Nøttestad *et al.* (2002) described 184 behavioural events, with an event occurring every 8.3 minutes, on average. These adaptive changes allow herring to adjust to the prevailing conditions. Predators are a natural stressor to herring and the approach of a predator causes an antipredator response such as “split”, “herd”, “fountain” or “dive” (Nøttestad and Axelson, 1999). The latter has been seen when a saithe school was observed attacking a herring school, forcing the school to dive to 150m, increasing the swimming speed and incurring energetic costs (Pitcher *et al.*, 1996).

The avoidance behaviour herring show towards predators in many ways resembles their response to fishing gear, in this case purse seines. Splitting schools and “panic swimming” are behavioural responses to visual contact with the net, as well as sound from the fishing vessel (Misund, 1993). These initial physical stressors are the start of a cascade of stressors that herring are exposed to in a purse seine. Crowding stress occurs during the final stages of hauling, just before slipping occurs and thus is the main focus of this thesis.

## 1.2 Stress response

When fish are exposed to stressors, such as crowding, a stress response is initiated as their homeostatic state is threatened (Chrousos, 1998). A stress response can be categorised into primary, secondary and tertiary responses (Barton, 2002). The primary stress response is a neuro-endocrine response that involves an increase in the catecholamines followed by a rise in plasma cortisol levels (Wendelaar Bonga, 1997; Barton, 2002). Cortisol is frequently used as a key physiological indicator of stress (Wendelaar Bonga, 1997). Herring have been shown to have elevated cortisol levels following high density crowding (Tenningen *et al.*, 2012) and these high levels remained for four days after crowding occurred (Figure 2).

The elevated cortisol and catecholamines act upon target organs within the fish to produce a secondary stress response. For example, blood flow to the gills and osmolarity increases, and glycogen is broken down increasing glucose. Glucose is rapidly utilised, as maintaining a stress response is energetically costly (Martinez-Porchas *et al.*, 2009; Santos *et al.*, 2010). There may also be increased activity of white swimming muscle causing the fish to respire anaerobically, increasing the concentration of lactate (Wendelaar Bonga, 1997). This is shown in herring by increased lactate concentrations during crowding (Figure 2) (Tenningen *et al.*, 2012).

Finally, the tertiary stress response is a whole-animal change in performance, an example of which is a change in behaviour (Barton, 2002). Behaviour has been shown to be a sensitive indicator to physiological and biochemical changes that occur in response to stress but may also be a direct neurological response to a stressor (Schreck *et al.*, 1997). Initially the response may be adaptive in order to increase the probability of survival by avoiding or removing the stress exposure. However if the stressor is overly severe or prolonged and cannot be avoided or removed then behaviours start to deviate from the norm, decreasing the probability of survival (Schreck *et al.*, 1997). Changes in behaviour are an animal’s first line of defence to adverse conditions (Schreck *et al.*, 1997) and are fast, easily observed responses, making them good indicators of welfare (Huntingford *et al.*, 2006; Martins *et al.*, 2012). The behavioural responses of herring to crowding stress and the deviations from behavioural norms are the focus of this study.

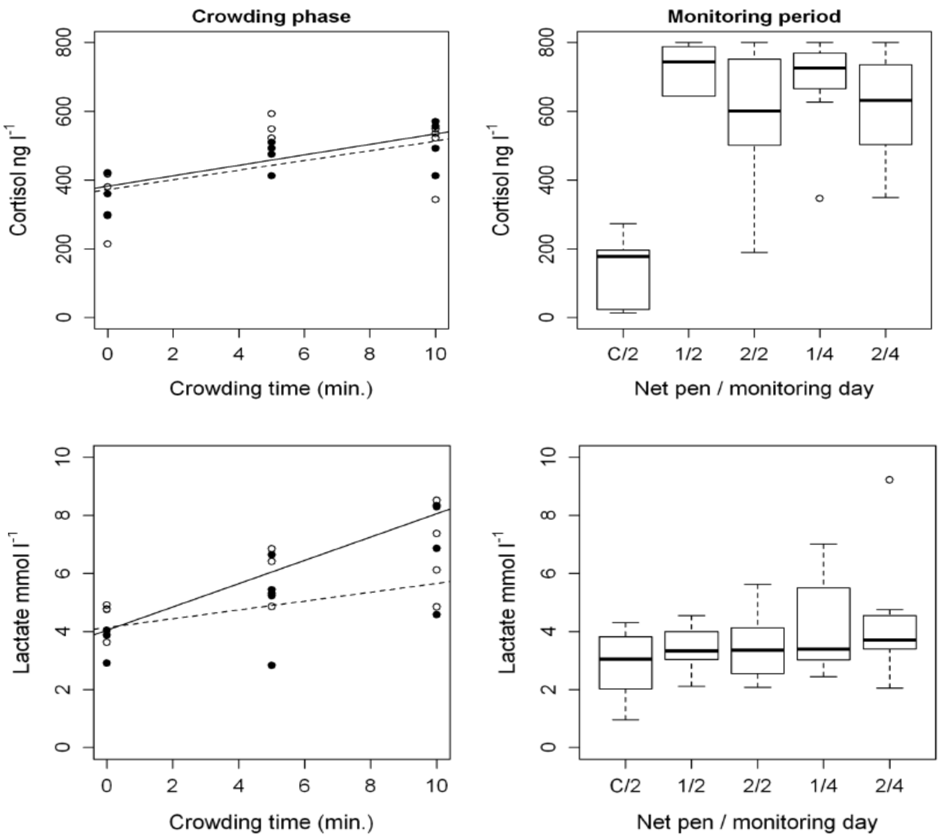


Figure 2: Cortisol & lactate levels in the blood during the crowding phase and during the monitoring period in the two crowded cages and in the control cage (C) on the second day of monitoring. In the graphs on the left, filled circles and solid lines represent net pen 1, and open circles and broken lines represent net pen 2 (Tenningen *et al.*, 2012).

Change in swimming activity has been shown to be a general behavioural indicator of stress (Schreck *et al.*, 1997). An increase in activity has been seen in herring exposed to hypoxia (Herbert and Steffensen, 2006) and scale loss (Olsen *et al.*, 2012), as well as mackerel exposed to thermal stress (Olla *et al.*, 1975). Whereas a decrease in activity was seen in sardine exposed to crowding (Marçalo *et al.*, 2013) and cod exposed to hypoxia (Schurmann and Steffensen, 1994).

Another behavioural indicator of stress is loss of orientation (Davis, 2002). During optimal conditions herring maintain their school structure by having a repulsion zone around them, preventing neighbours from being too close and thus giving them space to quickly change direction in response to a predator (Partridge *et al.*, 1980; Gueron *et al.*, 1996). However at high crowding densities the fish are forced within each other's repulsion zone. This can be an acute stressor, setting off a stress response, which may result in reduced ability for an individual to orientate itself with its neighbours. This can result in the organised structure within the school being lost with fish showing individual rather than collective behaviours. One of the characteristics of schools is that the fish are polarised, i.e. all orientated the same way (Figure 3a) (Shaw, 1978). A loss of synchronized orientation, depolarisation, (Figure 3b) could therefore be a behavioural indicator of when the welfare of the fish has been compromised.

(a) Polarised

(b) Depolarised

Figure 3: Expected orientation of: (a) a polarised and (b) a depolarised herring school

## 1.3 Aims and Objectives

Swimming activity and orientation could be two behavioural indicators of stress post-crowding. A detailed analysis of these was carried out using a number of behavioural metrics, involving both the behaviour of individuals and the relationship between neighbouring fish. Analyses were carried out on video footage/sequences from survival experiments, as outlined by Tenningen *et al.* (2012), and of herring in the wild. I aim to determine whether there is a relationship between behaviour and post-crowding mortality rates, over a range of crowding densities. Behavioural indicators have an advantage over physiological indicators of stress as they are less intrusive and could give a more rapid assessment of the welfare of the fish. If behaviour can be used to show the welfare status of the fish, then crowding density and time in the net may be controlled to minimise mortality.

The objectives of this study are thus:

1. To analyse whether the behaviour of herring differs when fish are exposed to different crowding densities.
2. To analyse whether the behaviour of herring changes over the experimental period.
3. To assess captivity effects through comparing the behaviour of herring in wild footage with that from the survival experiments.
4. To assess whether behaviour can be used as an indicator of stress in herring and whether there is a dominant behavioural metric for indicating stress.
5. To critically evaluate the data collection and analysis methods used and make recommendations for future studies.

# 2. Materials and Methods

## 2.1 Data collection

The data used in this thesis were collected from two sources:

1. Slipping mortality assessments by the Institute of Marine Research as part of project no. 82168, entitled: “Overleving av pelagisk fisk etter trenging i not” (Survival of pelagic fish after crowding in a purse seine), generated the mortality data, as well as video sequences of herring post-crowding; and

2. Video sequences of wild herring from Are Pilskog/Blåst Film AS, Norwegian Sea (between Buagrunnen and Svinøy Fyr).

Data from the mortality assessments were collected from 12th- 23rd March 2012 in the Norwegian fjords just south of Haugesund (Figure 4).

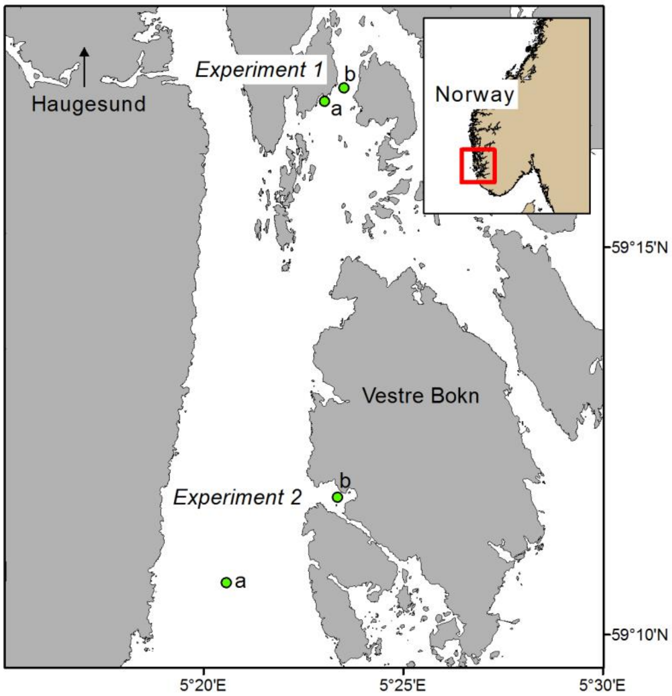


Figure 4: Map showing location of where - (a) the fish were caught and (b) the cages were located for Experiments 1 & 2.

Two experiments were conducted each consisting of a control and three trials. Both experiments lasted six days, the first experiment from 12th-17th March, and the second experiment from 18th-23rd March. The coastal seiner Sjonglør SF-51-SU took the catches in both experiments and the vessels Sjarmør SF-17-SU and Endre Dyrøy H-15-F assisted in handling catches and performing the crowding simulations.

The methodology for these experiments was originally developed for mackerel by Huse and Vold (2010) and involved setting a purse seine around a fish school, hauling the net approximately halfway in and then decanting the fish through a transfer channel and into a cage (Figure 5).

The transfer channel and cages were made of the same netting as the bunt of the purse seine and the cages had a 12m diameter and depth. A weight was attached to the bottom of each cage to help maintain the cages shape. Each purse seine haul filled four cages, one kept as a control and three used for crowding experiments. Each cage had an estimated 1-3 tonnes (Table 1) of herring transferred from the purse seine before the transfer channel was closed. This large variation in the amount of fish was difficult to avoid as the number of fish entering each cage was estimated visually and controlled by manually opening and closing the channel, while avoiding physical contact or potentially stressful interactions with the fish. A more precise control of the transfer would have risked injuring the risk through physical contact and/or inducing unnecessary stress through rapid and disturbing movements of the netting channel. This variation in the number of fish within each cage was also not shown to affect the mortality results.

During the experiments crowding was simulated by lifting up the bottom of the cage, thus reducing the volume of water within the pen and increasing the fish density. Crowding densities ranged from 54-351 kg/m$^3$ (Table 2) with the highest corresponding to densities that could occur just prior to slipping. Experiment 1 contained low and intermediate crowding densities resulting in low mortalities, therefore crowding density was increased in experiment 2, as it was thought that the first experiment was too precautionary (Table 2). Crowding densities were initially determined visually by researchers and experienced fishermen, and quantified post-crowding by measuring the dimensions of the cage during crowding (depth, a, width, b, and length, c) and then estimating the volume, assuming it approximated the shape of a half-ellipsoid (i.e. $V = 4/3.\Pi.a.b.c$). Crowding was simulated for ten minutes, as this reflects the duration of slipping in a commercial fishery.

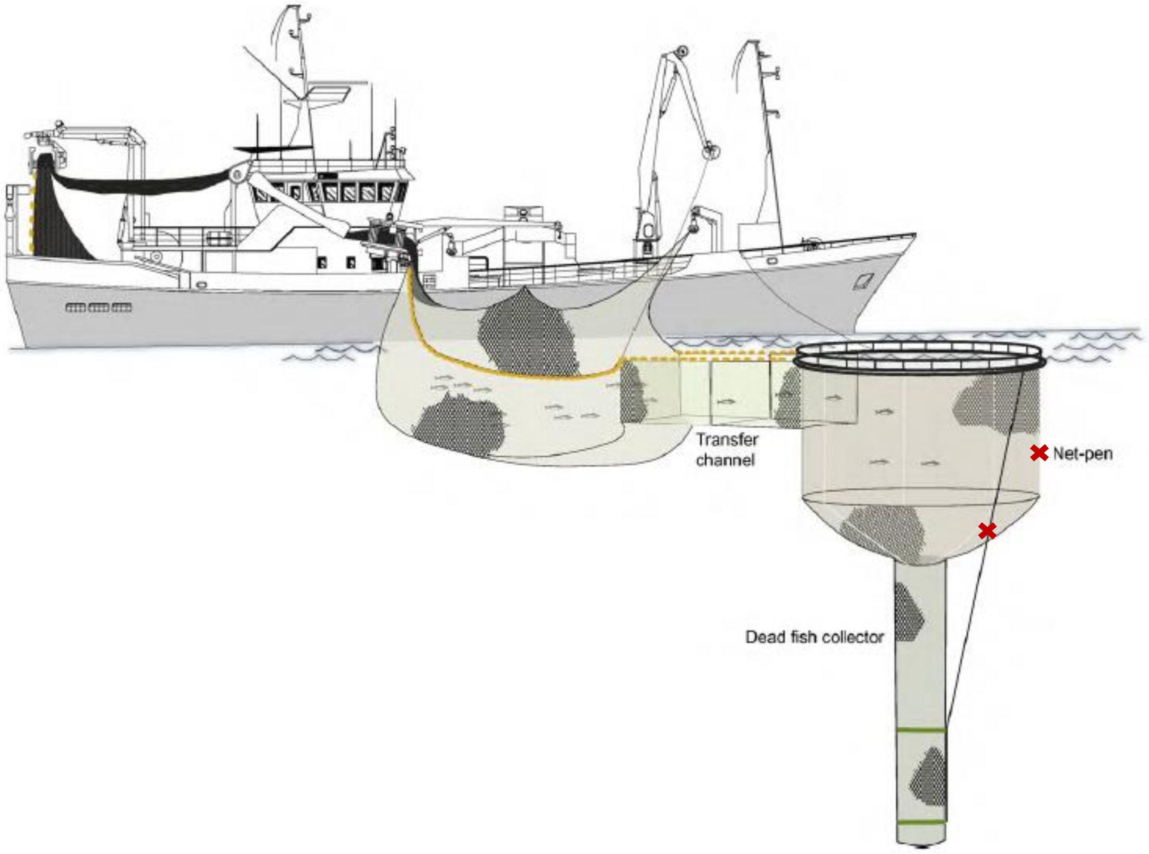


Figure 5: Survival experiment set-up. Fish were led from the purse seine to the net-pen through a transfer channel. Throughout the five day monitoring process dead fish were collected in bags at the bottom of the net. ✖ Indicates where video recordings were obtained. Illustration: Institute of Marine Research

After crowding, the cages were left for five days to monitor the mortality of the herring. An overall mortality rate was estimated over the entire monitoring period by recording the number of live and dead fish upon termination of the experiment; where dead fish were collected in bags at the bottom of the cage (Table 2). Daily mortality rates were not taken as it was not possible to count the dead fish in the bags without causing a lot of stress to the fish.

Table1: Table showing the number of individuals in each cage (N) the total biomass of fish (kg) and the mean length of the dead and alive fish (cm).

| Experiment | Cage | N | Biomass (kg) | Mean length (cm/fish) |
|---|---|---|---|---|
| 1 | Control | 5136 | 1221 | 32.4 |
| | Trial 1 | 11137 | 2710 | 32.3 |
| | Trial 2 | 13989 | 3382 | 33 |
| | Trial 3 | 3807 | 917 | 32.4 |
| 2 | Control | 11239 | 2714 | 32.5 |
| | Trial 1 | 8577 | 1938 | 32.5 |
| | Trial 2 | 10838 | 2430 | 32.5 |
| | Trial 3 | 7825 | 1769 | 32.6 |

Table 2: Table outlining the experimental setup and the corresponding crowding densities and mortalities. The order of the cages indicates the order they were filled.

| Experiment | Cage | Crowding density | | Mortality |
|---|---|---|---|---|
| | | (kg/m$^3$) | (fish/m$^3$) | |
| 1 | Trial 3 | 200 | 826 | 0.050 |
| | Control | 1.5 | 6.2 | 0.011 |
| | Trial 1 | 54 | 225 | 0.013 |
| | Trial 2 | 160 | 658 | 0.016 |
| 2 | Trial 2 | 351 | 1565 | 0.278 |
| | Trial 1 | 247 | 1092 | 0.222 |
| | Trial 3 | 263 | 1161 | 0.224 |
| | Control | 3.3 | 13.6 | 0.046 |

### *2.1.1 Weather conditions*

There was fog, light rain and little wind during transfer and crowding for experiment 1. For experiment 2 the weather started out with sunshine and almost no wind, however as the day progressed the wind increased to a gale. During termination of experiment 2 the wind was very strong and at the borderline of working conditions. The sea temperature was 6.1°C in experiment 1 and 6.2°C in experiment 2.

### *2.1.2 Catch and cage locations and timings*

For experiment 1 the catch was taken west of the island Høvring at 59°16’50”N 05°22’41”E (Figure 4) whilst it was dark (03:05-03:30) and transferred to the cages at 07:30. All four pens were filled, starting at 09:45. The cages were located almost on the same place as where the fish were caught, 59°16’59”N 05°22’59”E (Figure 4). Crowding was carried out at 17:09-17:22 (trial 3), 20:01-20:12 (trial 1), and 21:51-22.01 (trial 2) the same evening.

For experiment 2 the catch was taken at 59°10’36”N 05°20’32”E (Figure 3) on 17th March, transferred to a towing pen and towed to a bay called Grønnestadvågen, 59°11’44”N 05°23’15”E (Figure 4). Towing the test population is not ideal in such a survival assessment, as it has the potential to induce additional stress, fatigue and injury. However, every care was taken to minimise such stressors by using well tried, traditional techniques used by coastal purse seine fishermen when handling and storing catches. The fish were towed approximately 1.8 nautical miles at a speed of around 1 knot. Towing would therefore have lasted approximately 2 hours. The fishermen ensured a slow speed during towing and paid close attention to the behaviour in order to minimise stress.

The fish were left for 1 day in the towing pen in order to allow recovery from towing and on 18th March the fish were transferred to the cages, starting at 12:40. All four pens were filled by 15:00. Crowding was carried out at 15:33-17:43 (trial 1), 19:56-20:06 (trial 2), and 20:58-21:08 (trial 3).

## 2.2 Data Analysis

### *2.2.1 Video collection*

Video footage was obtained from different sources, within the survival experiments and from herring filmed in their natural environment. Within the survival experiments footage was taken during different stages: transfer, crowding in the cage and post-crowding daily during the monitoring period. Footage taken during transfer between the purse seine and cages were only taken sporadically to assess the suitability of the fish for the experiments, and to try to ensure the fish swam calmly through the channel, maintaining their “normal” schooling behaviour. Post-crowding the video footage was more systematic and therefore this is what was used for analysis. Video footage was also available from survival experiments conducted in 2008, 2009 and 2011, however only the 2012 videos were used. This is because 2012 had the most complete dataset and the videos showed the highest quality as recordings were taken directly at the cage. The first footage used was on the day of crowding (day 0) after the crowding event and then daily until the termination of the experiments 5 days after crowding. Unfortunately no video was taken during the final termination day for experiment 1 and therefore the last video available is 4 days after crowding. The wild video footage was obtained from Are Pilskog and was raw footage from the film ‘Havets sølv’ recorded by a scuba diver.

### *2.2.2 Camera specifications*

The video footage from the survival experiments were collected using an underwater 360° pan and tilt camera system, BENNEX BC-300 series (Figure 6, www.bennex.no).

The camera has an angle of view of 72 ° in the water and was designed with a robust construction and the ability to produce high quality colour pictures (752 x 582 Effective pixels) making it suitable for behaviour studies.

The camera was attached to a cable and was lowered down the side netting of the cages. The movement and pan and tilt were controlled on the surface of the cage using a control

Figure 6: BENNEX BC-300 series pan and tilt underwater camera system.

panel and a video screen provided a live feed. The camera was used to take a mixture of footage from the side and the bottom of the cages (Figure 5) of which some were vertically pointing towards the surface and some were horizontal to the surface.

### *2.2.3 Cataloguing video*

Short sequences of video were extracted from the 14 hours of raw video. The sequences were categorised into vertical and horizontal camera angles (Figure 7). The horizontal footage was then further categorised into sequences shot midway down the cage to those shot at the bottom of the cage. Only the sequences shot midway down the cage were used in this study. The vertical footage was more limited and the majority was taken from the bottom of the cage so no further categorisation was needed. There was no vertical footage available for the wild footage. The sequences were catalogued according to day, cage and camera position (Appendix 1).

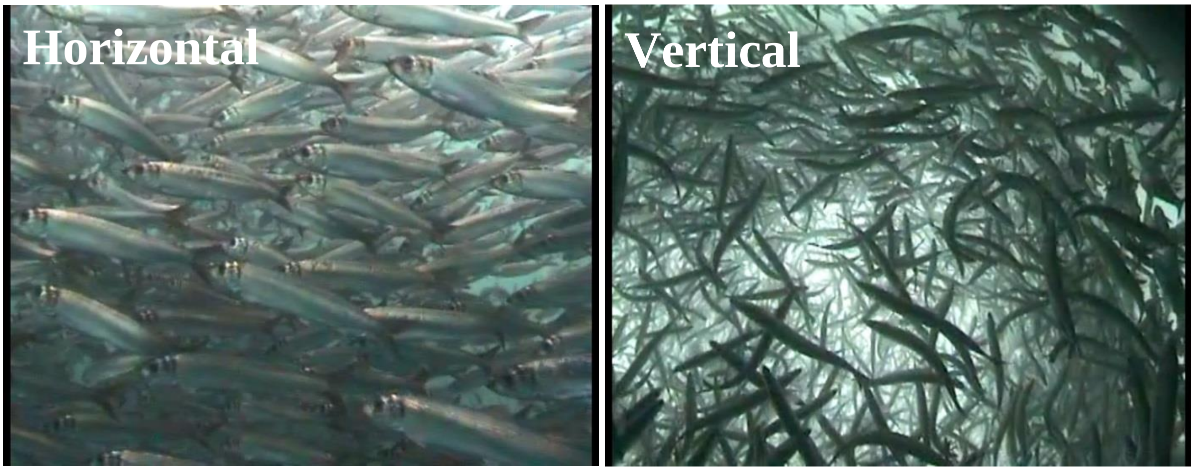


Figure 7: Example frames of the horizontal and vertical camera angles selected.

These video sequences were then converted to images using Adobe Premiere Pro, taking 24 frames per second. The sequences were then randomised in order to minimise the source of error due to observer bias when carrying out the analysis and the random numbers recorded in the sequence catalogue to refer to post-analysis.

### *2.2.4 Analysis software*

Images were analysed using ImageJ 1.47t software (http://imagej.nih.gov/ij). The software is written in Java and is open source. As there were many fish in each image (Figure 8) and a large amount of camera movement within the data, all the analysis was carried out manually and ImageJ was chosen as it had all the features that were needed to perform this (Figure 9). It is also a flexible program with a possibility to expand through the use of plugins (Figure 9). The plugin "Grid" was installed for use in this thesis to increase efficiency when selecting fish though use of grid coordinates (http://rsbweb.nih.gov/ij/plugins/grid.html).

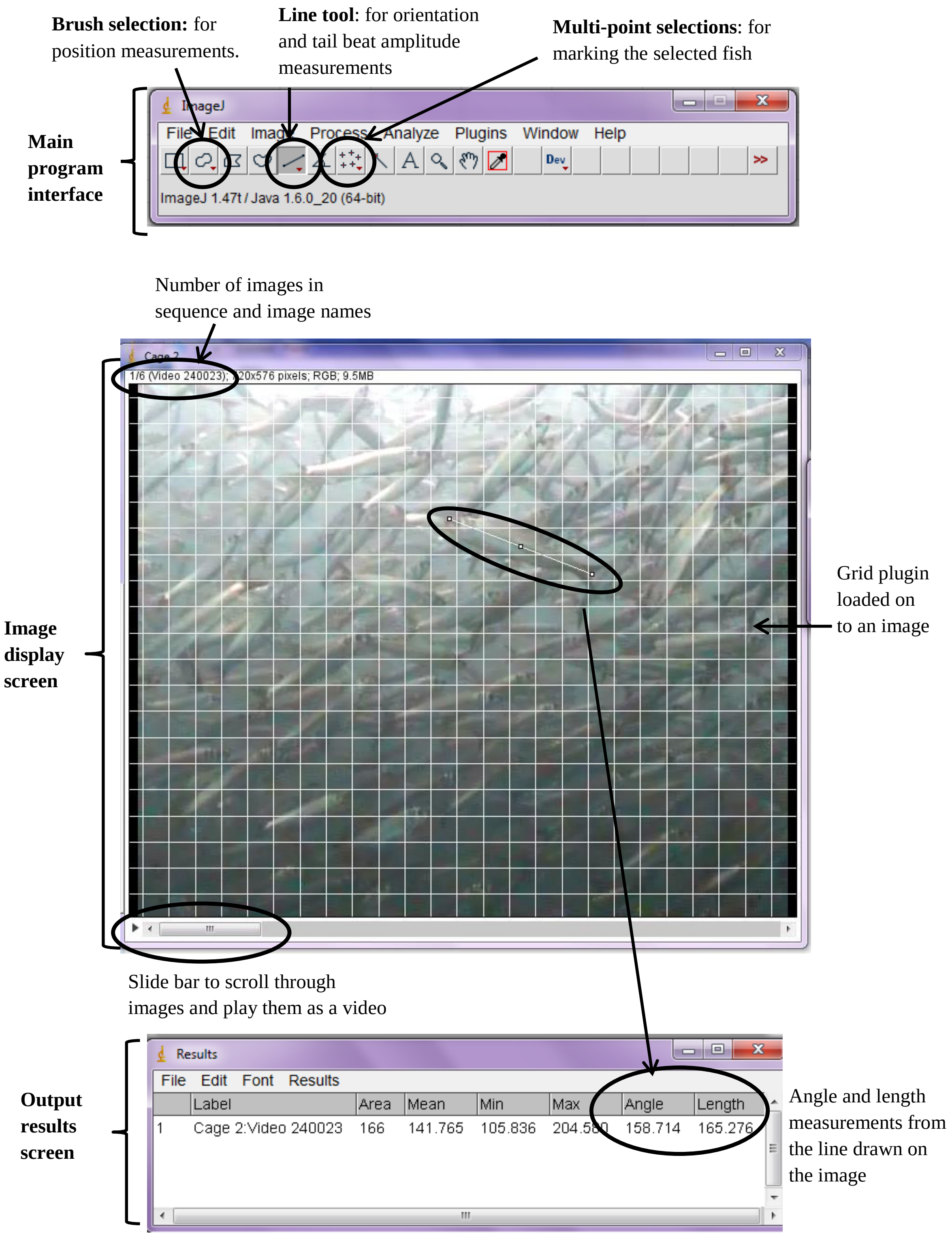
### *2.2.5 Selection of fish*

Due to the high numbers of fish within each image frame a subsample was selected to enable data analysis. Ten individual fish were chosen at random by overlaying a grid on to the image and randomly generating ten grid coordinates using R. The fish lying closest to the centre of the chosen grid square was selected and marked using the Multi-point tool in Image J. Each fish was given an identification number from 1-10, the grid was then removed from the image to improve clarity (Figure 8).

If the fish selected was at the edge of the initial frame and there was a high likelihood that it would not remain in the subsequent frames a new fish was selected.

Figure 8: Example frame showing ten randomly selected fish

# Image J



|  | Label | Area | Mean | Min | Max | Angle | Length |
|---|---|---|---|---|---|---|---|
| 1 | Cage 2:Video 240023 | 166 | 141.765 | 105.836 | 204.580 | 158.714 | 165.276 |

Figure 9: ImageJ 1.47t user interface with some of its features annotated

## 2.3 Swimming activity

### *2.3.1 Tail beat frequency*

Due to a large amount of camera movement, and lack of reference points in the images, swimming speed could not be measured directly through distance travelled or changes in x, y co-ordinates. Instead the swimming activity was first measured by counting the tail beat frequencies. The sequences where the camera angle was vertical were used for this, as the tail beats could be seen more clearly than in the horizontal footage. This meant tail beat frequency and the other swimming activity metrics could not be taken on the wild sequences.

A sequence duration of 200 frames (8.3 seconds) was used, in order to maximise the time that the fish's activity could be monitored. Only one sequence was taken per cage per day. Ten fish were randomly selected and the number of tail beats was counted for each of the ten fish over the 200 frame sequence.

### *2.3.2 Tail beat amplitude*

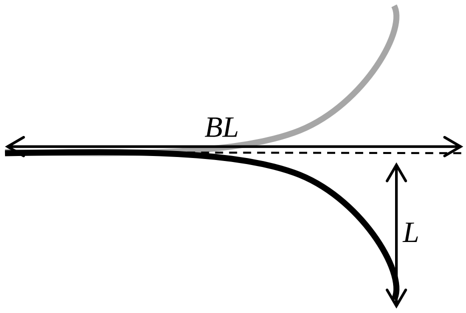


Figure 10: Calculating tail beat amplitude by measuring the length of half the tail beat (*L*), in pixels. The body length (*BL*) in pixels was measured and proportion was calculated using the formula *L/BL*.

The second metric for analysing swimming activity was tail beat amplitude. This was also measured in each sequence on the same ten individual fish as the tail beat frequency was measured. The length of the fish was first measured in pixels, using the line tool on ImageJ. The distance from the lateral most excursion of the tip of the tail to the centre axis of the fish was then measured in pixels (Figure 10). The recorded tail beat amplitude was then divided by the length of the fish to give the tail beat amplitude as a proportion of the length. This was done for at least two tail beats on each fish and more where possible. The average tail beat amplitude for each fish was then calculated.

### *2.3.3 Swimming mode categorisation*

Three sets of swimming modes were identified from the data: (1) gait, (2) turning (3) tail beat and split into the steady and alternative swimming modes (Table 3).

Table 3: Categorisation of three swimming modes split into steady and alternative

| Mode | Steady | Alternative |
|---|---|---|
| **1) Gait** | Steady swimming | Kick & glide |
| **2) Turning** | <90° | >90° |
| **3) Tail beat** | Full | Half |

These swimming modes could not be measured quantitatively so categorisation was used. Each of the three behaviour sets was identified as being either present or absent. All three behaviour sets could be present at the same time, however it was not possible for the fish to show both behaviours within each set (e.g. half and full tail beat). The categorisation was carried out on the same fish that were used for the tail beat frequency and amplitude measurements.

1. *Gait*

If the fish showed a short burst of tail beats followed by gliding for over half the sequence duration (100 frames), then kick & glide was marked as present. If this was not present then it was assumed that the fish was steadily swimming, so steady swimming was marked present.

2. *Turning*

If the fish turned more than 90° throughout the sequence, then turning was marked as present. If the fish turned less than 90°, turning was marked as absent and it was assumed that the fish was swimming along a relatively straight path.

3. *Tail beat*

If the fish was not completing full tail beats (the tail would return to the centre axis but not pass to the other side of the body) for over half of the sequence duration, then half tail beats were marked as present. If not, then full tail beats were marked as present (the tail would pass both sides of the centre body axis).

## 2.4 Orientation

The second group of behavioural metrics were related to orientation of the fish. Both horizontal and vertical orientation was measured in order to give a more complete image of orientation in a 3D environment.

### *2.4.1 Nearest neighbour distance (NND) & horizontal orientation*

Vertical images were again selected for this metric. Wild images could not be used.

One frame from each day and each trial was selected to carry out this analysis. From each frame ten fish were selected using the predefined method (see section 2.2.5), however to ensure fish were approximately the same distance from the camera an additional selection criteria was applied whereby the selected fish were of approximately the same pixel length. If the selected fish was larger or smaller (approximately 10% of body length) than the other fish, a new fish was randomly selected. Using this method and assuming all fish are the same size means that the ten fish selected were about the same distance from the camera. The nearest parallel neighbour to each of the fish was then selected. Only parallel nearest neighbours were selected to reduce the effect of a circular cage (Figure 11).

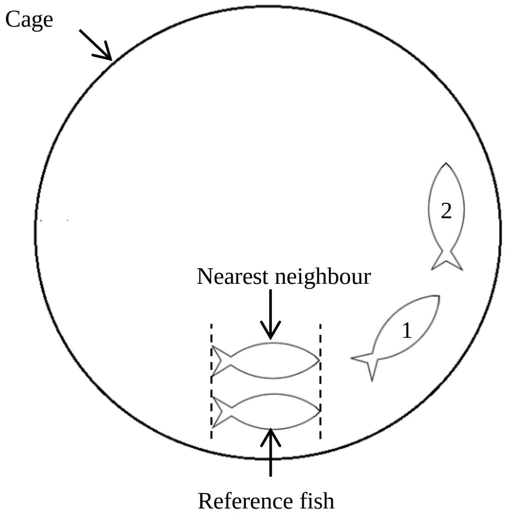


Figure 11: Due to the circular nature of the cage the fish have to constantly turn. Fish 1 and 2 are orientated in the same direction as the reference fish in relation to the side of the net. However from measuring their individual angles they show a large difference in orientation between them. To reduce this problem the nearest neighbour fish parallel to the reference fish was selected.

The angle of each of the ten fish and their corresponding nearest neighbour were measured using the line tool on ImageJ and the x and y coordinates were recorded at the mid-length of each fish. Using the x and y coordinates, the 2D distance between each of the ten fish and their nearest neighbour (NND) could be calculated using Pythagoras' Theorem. In order to make the distances between the ten fish comparable, it was important to select fish that were approximately the same distance from the camera.

The deviation in angle of the nearest neighbour from the reference fish was calculated in degrees. If the difference was large then the fish were poorly aligned and if it was small they were well aligned. The angle was therefore converted to an alignment measurement ranging from 0-1. If alignment = 0 the fish are not at all aligned (angle=180°) and if alignment=1 then the fish are perfectly aligned (angle=0°).

The alignment could then be compared to the distance between the nearest neighbour and reference fish to see how alignment changes with distance.

### *2.4.2 Vertical Orientation*

Horizontal image sequences midway down the cage and the wild image sequences were selected for this metric. Due to this the vertical orientation was measured on a different set of ten fish to those used for swimming activity & horizontal orientation.

The sequences consisted of a series of 60 frames (2.5 seconds duration) with at least two sequences per cage per day. From the wild footage 13 sequences were selected. Preliminary measurements indicated that the majority of fish pass through the cameras field of view in five seconds; therefore this was defined as the minimum time between sequences required to ensure independence between sequences. Sequences were therefore separated by a minimum of 120 frames.

Each sequence was given a unique sequence identification number. Within each sequence, one frame in every six was analysed, giving a total of 10 analysed frames.

In the first frame ten fish were randomly selected. If the fish selected was not swimming approximately parallel to the camera a new fish was selected as measurements were only taken in 2D so this minimised the 3D error.

On the first fish a line was drawn from the tail fork to the head, using the Line tool on ImageJ. The angle of the fish, relative to the horizontal baseline within ImageJ was recorded (Figure 9). The same fish was then tracked over the ten frames and its orientation measured in the same way, before repeating the method for the other nine fish.

### 2.4.2.1 Variance

As vertical orientation is what is being measured the raw angles were converted from 0 to ±180° to 0 to ± 90° (Figure 12). The variance was then calculated for the angles of the ten fish in each frame using the formula:

$$\alpha = \frac{1}{N}\sum(x_i - \bar{x})^2$$

$N$=number of fish (10)   $x_i$=angle of fish $i$,   $\bar{x}$ =mean angle of the 10 fish

Variance gives a measure of how far the values deviate from the mean, therefore in this case the smaller the variance the more aligned the ten fish are.

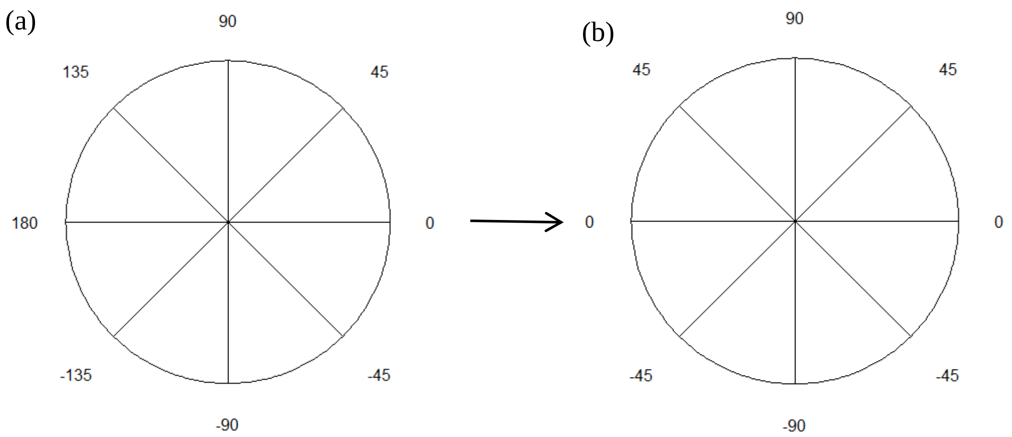


Figure 12: Changing the axis post-analysis: from 0 to ±180° prior to adjustment (a) to 0 to ± 90° after adjustment (b).

### 2.4.2.2 Extreme angles

As well as looking at the variance between fish, the raw angles were categorised into ‘normal’ and ‘extreme’ angles. The extreme angles were defined as between 45° and 135° and -45° and -135° (Figure 13). Extreme orientation was marked as either present or absent for each fish.

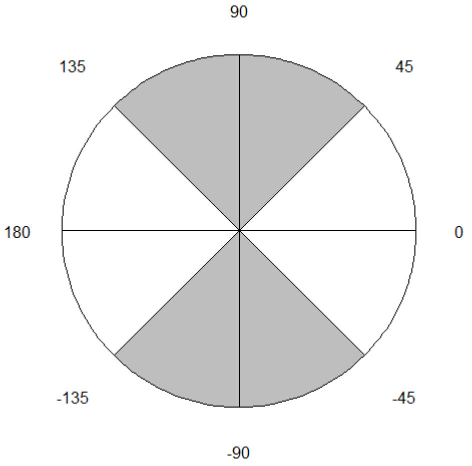


Figure 13: Extreme angles marked grey (-45° to -135°) and (45° to 135°).

## *2.5 Statistical analysis*

The statistical program R version 2.15.1 (R Core Team, 2012) was used for all statistical analyses and plotting. The two control cages were grouped together for plotting and analysis and the crowding density was set to 0 kg/m$^3$ for the control group. In all analyses a maximal model was fitted first. The model was then simplified by removing the non-significant terms. The model with lowest AIC was selected as the final model. All statistical tests assumed a 0.05 significance level.

For analysing tail beat frequency analysis of covariance (ANCOVA) (Tabachnick and Fidell, 2007) was used using day as a factor and crowding density as a continuous variable. Analysis of variance (ANOVA) (Gravetter and Wallnau, 2000) was also used to compare if tail beat frequency changed from the start to the end of the experiment when days were grouped. Regression analysis (Gravetter and Wallnau, 2000) was used for comparing mortality and tail beat frequency. Binomial generalised linear models (GLMs) (Dobson, 1945) were used to model the swimming behaviours. Linear mixed effect models (LMEs) (Oberg and Mahoney, 2007) were used in the analysis of tail beat amplitude. In order to test whether tail beat amplitudes are affected by swimming mode an LME using maximum likelihood estimation was used, and performed on each mode each day separately in order to remove the need for Bonferroni correction, thus reducing type I errors.

Mixed Effect models or Generalised Linear Mixed Models (Breslow and Clayton, 1993) were fitted using Penalised Quasi-Likelihood (GLMMpql) (McCulloch, 2006) were used for orientation metrics. The sequence identification number was set as the random effect as some days have more than one sequence per cage.

Model and test outputs are shown in the Appendices.

# 3. Results

## 3.1 Swimming activity

### *3.1.1 Tail beat frequency*

Tail beat frequency was correlated with both crowding density (ANCOVA, $F_{1,339}=31.49$, $p<0.0001$) and observation day (ANCOVA, $F_{5,339}=3.06$, $p=0.0103$),with no interaction between the two (Appendix 2).

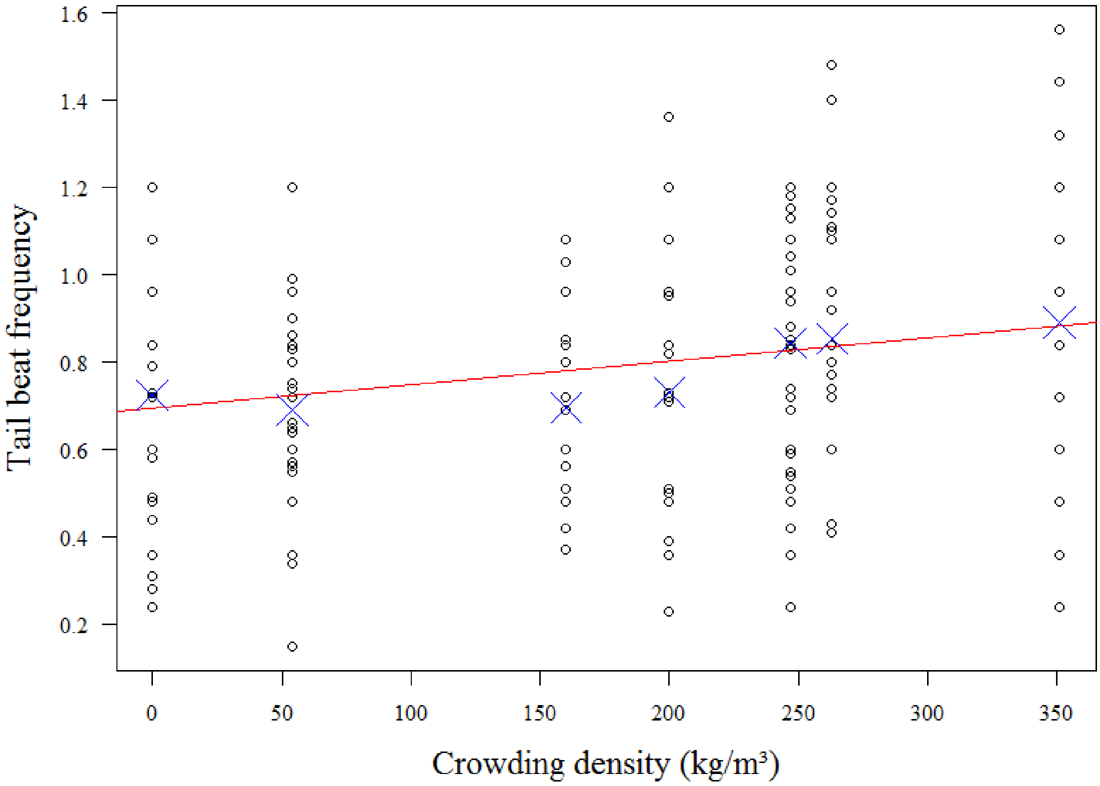


Figure 14: Raw tail beat frequencies over crowding densities ranging from 54-351 kg/m$^3$ and the control. Red predicted lines are from the linear model: lm(Frequency~Crowding) $R^2=0.11$, $F_{1,339}=31.5$, $p<0.0001$. Blue crosses represent means for each crowding density.

Tail beat frequency increases with crowding density (Figure 14). The lowest mean tail beat frequencies are found in the control ($\bar{x}= 0.72 \pm 0.20$ s.d., n=80) and lowest crowded (51 kg/m$^3$: $\bar{x}=0.69 \pm 0.20$ s.d., n=38) groups and the highest mean frequency at the highest crowding density of 351 kg/m$^3$ ($\bar{x}=0.89 \pm 0.28$ sd, n=60) (Figure 14).

Tail beat frequency was also correlated with day, with a decrease in frequency over time. This is particularly seen from day 2 to day 3 where the mean frequency decreased 8.5% (0.07 tail beats per second), and then remained lower at days 4 and 5 (Figure 15).

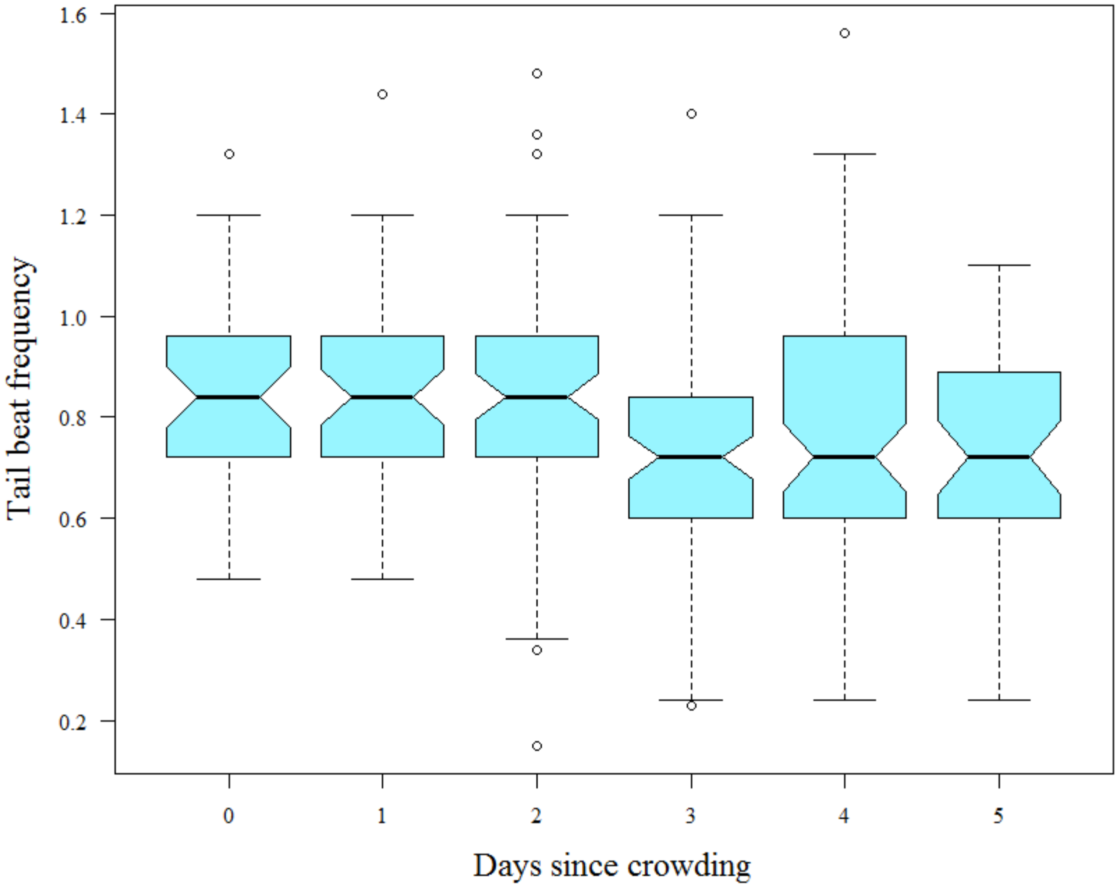


Figure 15: Boxplot showing tail beat frequencies (beats per second) from the day of crowding to 5 days afterwards (all crowding densities combined). If notches do not overlap there is a 95% certainty that the groups differ. Box= lower quartile (25%), median (middle line) and upper quartile (75%). Whiskers = 1.5x inter quartile range.

Figure 15 suggests two groups: days 0-2 (start of the experiment) and days 3-5 (end of the experiment), and therefore post-analysis grouping was carried out and tested to see if there is a significant difference between the start and end of the experiment (Figure 16). Although there was considerable variation, there was a significant decrease (of 10.7%, 0.09 tail beats) between the start (days 0-2: $\bar{x}$=0.84 ± 0.22 s.d.) and the end (days 3-5: $\bar{x}$=0.75 ± 0.25 s.d.,) of the experiment (1-way ANOVA, $F_{1,344}$=12.28, p=0.0005, Appendix 3) (Figure 16).

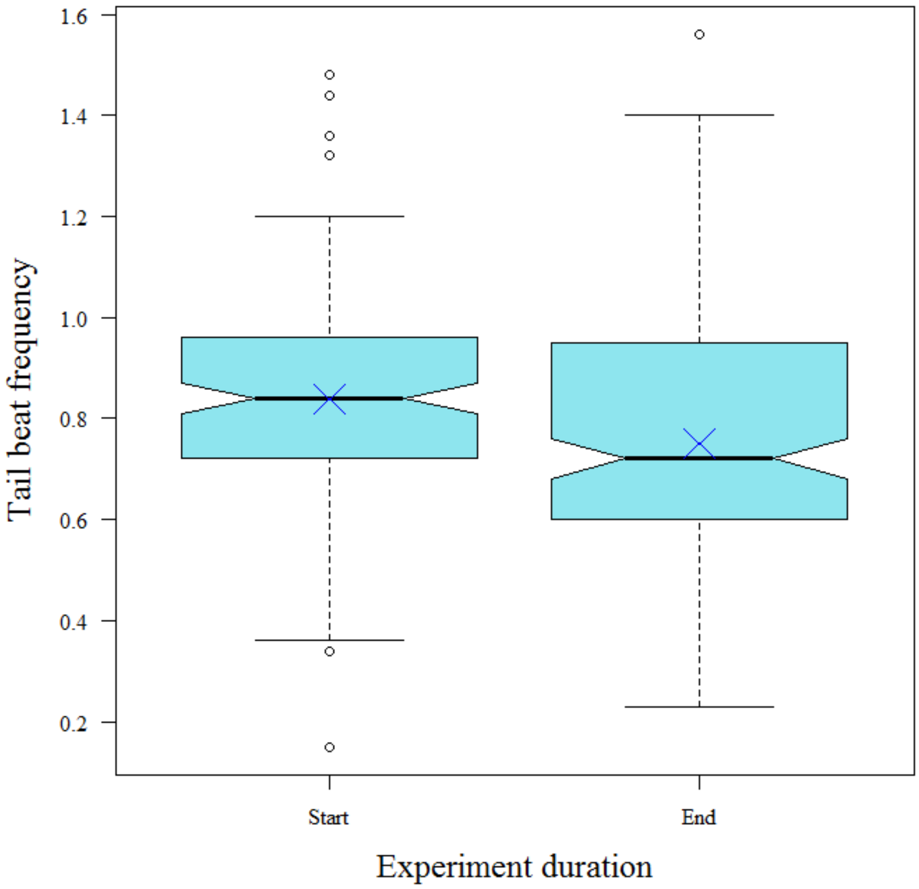


Figure 16: Boxplot showing tail beat frequencies at the start of the experiment (days 0-2) and at the end of the experiment (days 3-5) (all crowding densities combined). Blue crosses represent mean tail beat frequency. If notches do not overlap there is a 95% certainty that the groups differ. Box= lower quartile (25%), median (middle line) and upper quartile (75%). Whiskers = 1.5x inter quartile range.

However it is important to know if this decrease in tail beat frequency is significant across all crowding densities by looking at the change in tail beat frequency, at each crowding density, over the duration of the experiment. The highest crowding density (351kg/m$^3$) has the greatest decrease from the start (Day 0, $\bar{x}$= 1.0) to the end (Day 5, $\bar{x}$= 0.6) of the experiment (Figure 17) and has a significant difference over time (1-way ANOVA, $F_{5,54}$=5.54, p=0.0003). There is also a significant decrease in tail beat frequency at 200 kg/m$^3$ (1-way ANOVA, $F_{1,17}$=16.15, p=0.0009), however as data is only available for two days then no conclusions can be made (Figure 17). No other crowding densities show a significant difference in tail beat frequency over time (Appendix 4).

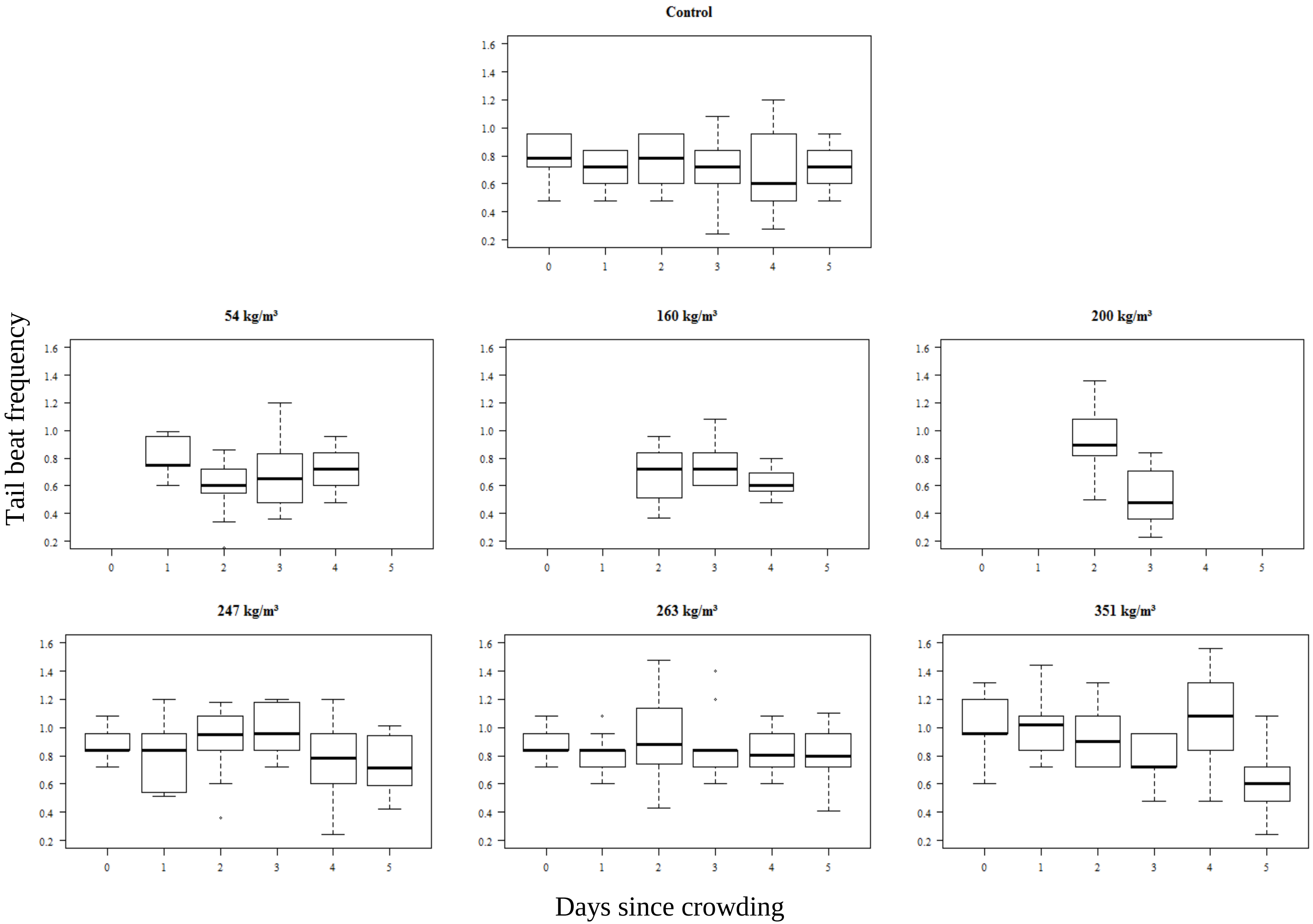


Figure 17: Change in tail beat frequency over days since crowding for control and crowding densities (54-351 kg/m$^3$). Box= lower quartile (25%), median (middle line) and upper quartile (75%). Whiskers = 1.5x inter quartile range.

#### *3.1.1.1 Tail beat frequency and Mortality*

Linear regression analysis shows a strong positive correlation between mortality and mean tail beat frequency ($R^2$=0.99, p=<0.0001, Appendix 5) (Figure 18). As tail beat frequency increased mortality also increased. The biggest increase for both mortality and tail beat frequency occurred between 200 and 247kg/$m^3$ where tail beat increased by 0.11 tail beats/second and mortality increased by 17%.

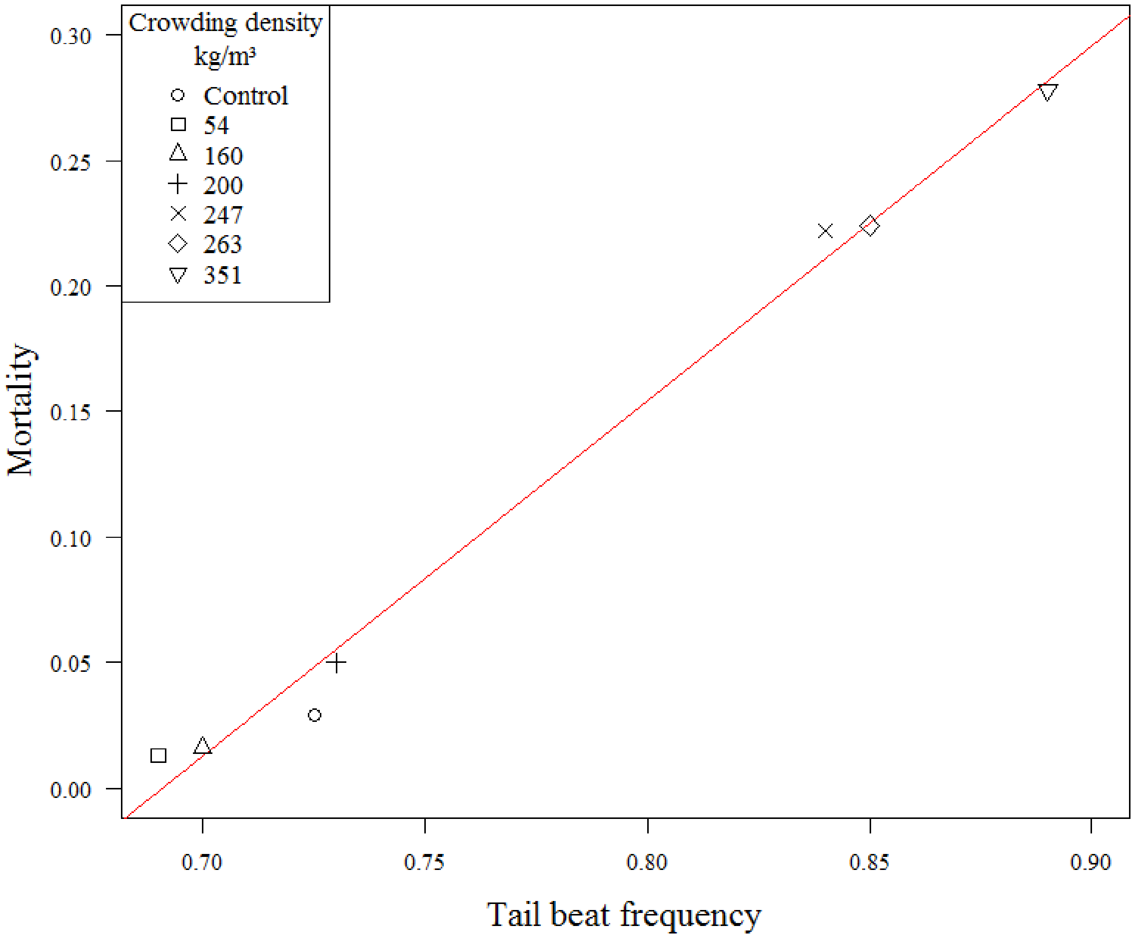


Figure 18: The relationship between mortality and mean tail beat frequency for each crowding density (all days combined). Linear regression line: Mortality=-0.98 + 1.41 x Tail beat frequency ($R^2$=0.99, p=<.0001) shown in red.

### *3.1.2 Swimming modes*

*Gait*

There was an apparent significant difference between the occurrence of kick and gliding/steady swimming between different crowding densities (GLM, p=0.0349, Appendix 6). The lowest occurrence of kick and gliding is found at a high crowding density (263 kg/m$^3$) and the highest kick and gliding behaviour at a low crowding density (54 kg/m$^3$, Figure 19a). However, there appears to be no consistent relationship as crowding increases (Figure 19a).

Over time there is no significant difference between the occurrence of kick and gliding and steady swimming with fish maintaining around a 40% kick and glide occurrence throughout the experiments (Figure 19a & 20a).

*Turning*

Turning >90° was more likely to occur when the fish were steadily swimming than when they were showing a kick and glide behaviour (Figure 19b). However, no significant relationship was found between the occurrence of turning at different crowding densities (Figure 19b) or over time (Figure 20b).

*Tail beat*

Half tail beats were more frequent when fish were showing a kick and glide behaviour than when they were steadily swimming (Figure 19c & 20c). Overall full tail beats were more common than half tail beats. There was no significant relationship between the occurrence of half or full tail beats at different crowding densities or over time.

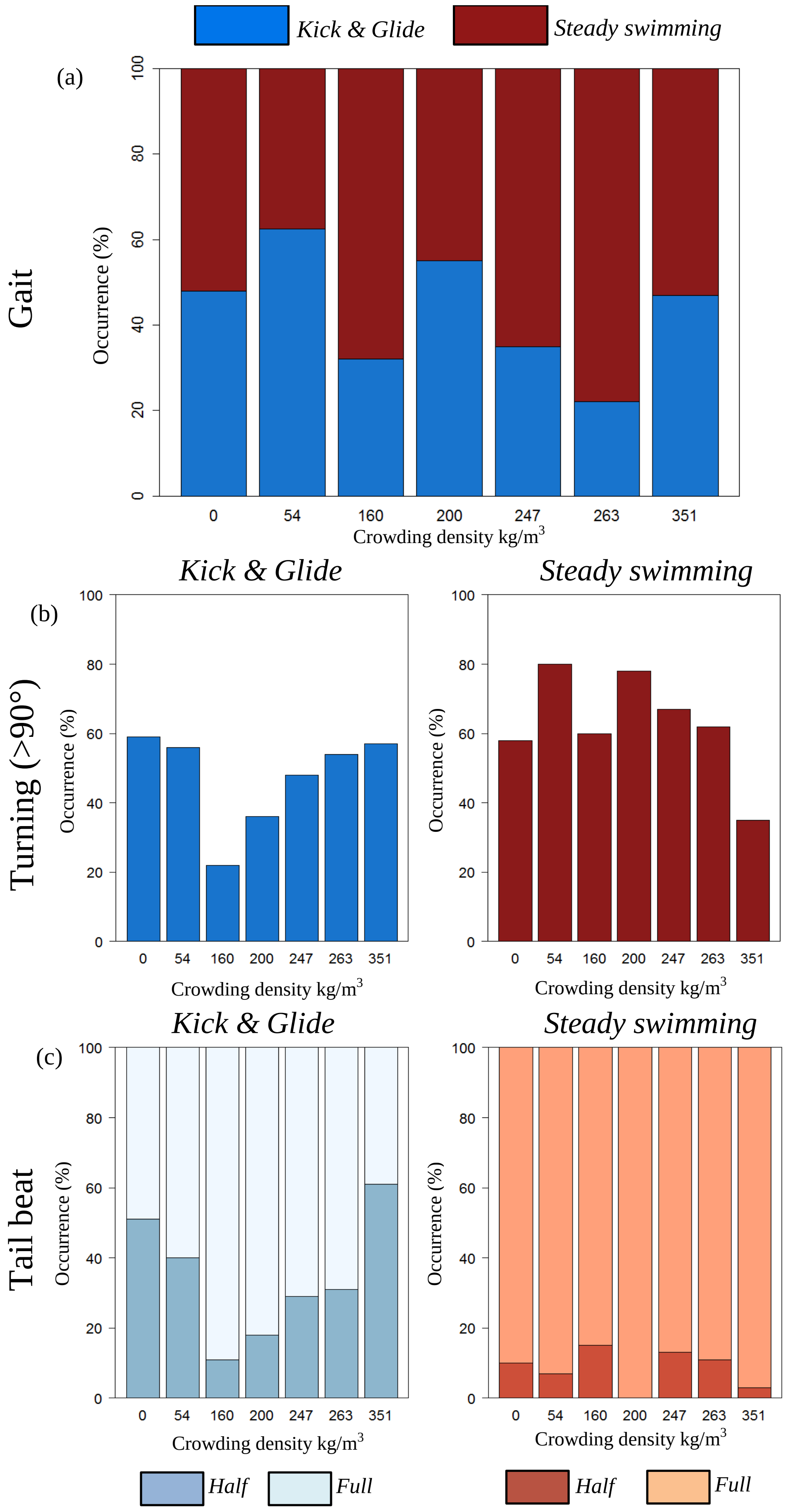


Figure 19: Occurrence of swimming modes over crowding densities (0-351kg/m$^3$). Turning (b) and Tail beats (c) broken down into gait (whether the fish were showing a kick and glide behaviour or steady swimming) (a).

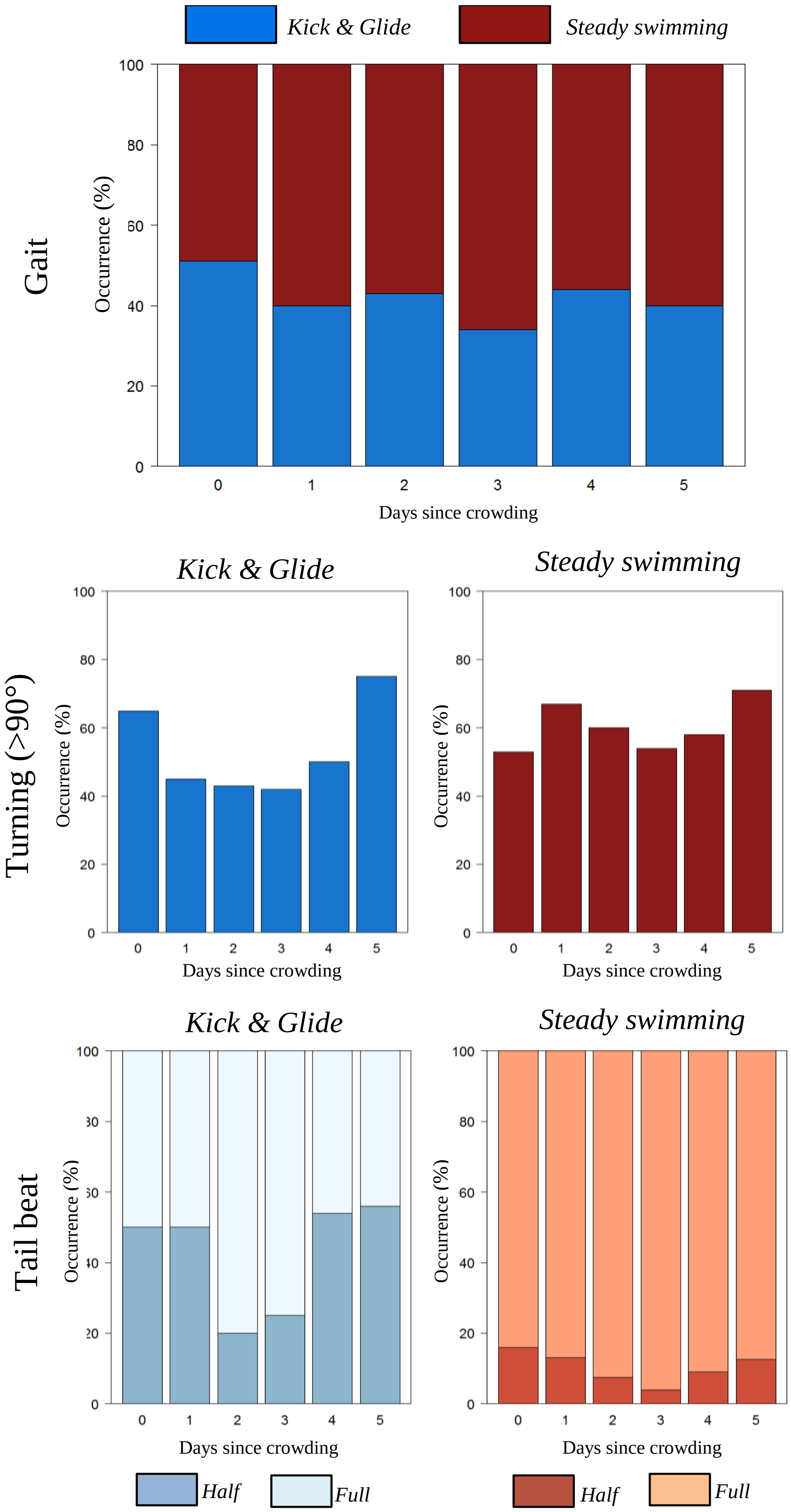


Figure 20: Occurrence of swimming modes over days since crowding. Turning (b) and Tail beats (c) broken down into gaits (whether the fish were showing a kick and glide behaviour or steady swimming) (a).

### *3.1.3 Tail beat amplitude*

Although there was no correlation between the tail beat amplitude and crowding density (Linear mixed effect model, $F_{1, 33}$=0.02, p=0.8889) or over time (Linear mixed effect model, $F_{5, 29}$=0.23, p=0.9472, Appendix 7), there were some clear differences when comparing the tail beat amplitude between the different swimming behaviours.

The largest difference in tail beat amplitude occurred on the day of crowding. The amplitude was then highest during kick & glide, in turning fish and in fish using half tail beats (Figure 21). There was however no correlation between tail beat amplitude and these swimming behaviours over crowding density.

*Gait*

Fish that show a kick and glide behaviour generally had a higher tail beat amplitude than fish steadily swimming, but a significant difference was only found at day 5 (Table 4). Day 0 appears to show a large difference in tail beat amplitude (Figure 21a), but the variation is large.

*Turning*

Tail beat amplitude was greater for fish that are turning compared to fish that are not turning except at day 5 (Figure 21b). This difference is significant at days 0 and 1 (Table 4).

*Tail beat*

The mean tail beat amplitude was generally greater when the fish swam using half tail beats than full tail beats except at day 3 (Figure 21c). There is a significant difference at days 0, 1 and 4 (Table 4). No correlation with crowding density was found.

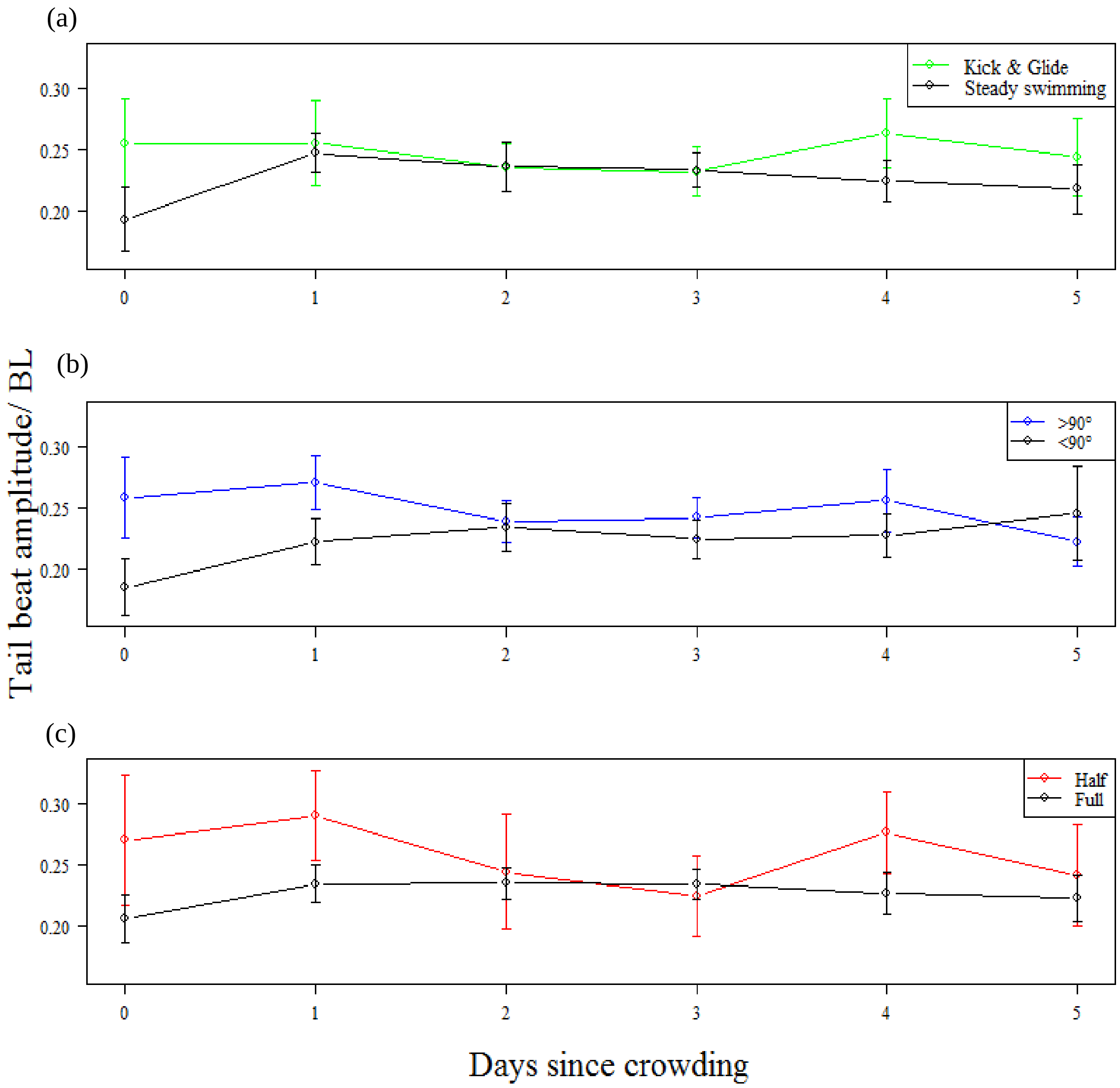


Figure 21: Change in mean tail beat amplitude over days since crowding (days0-5) for swimming modes: (a) Gait, (b) Turning, (c) Tail beat. Black lines represent the steady modes and coloured lines the alternative modes.

Table 4: Output from linear mixed effect model using maximum likelihood estimation for tail beat amplitude at each of the swimming modes: gait, turning, and tail beat. Each day tested separately. Significant relationships are shown in **bold**. (Appendix 8)

| Days since crowding | Gait | | Turning | | Tail beat | |
|---|---|---|---|---|---|---|
| | *F-value* | *p-value* | *F-value* | *p-value* | *F-value* | *p-value* |
| 0 | 1.91 | 0.1761 | **9.59** | **0.0038** | **6.67** | **0.0141** |
| 1 | 0.20 | 0.6559 | **9.76** | **0.0032** | **12.01** | **0.0012** |
| 2 | <0.01 | 0.9791 | 0.09 | 0.7624 | 0.28 | 0.5978 |
| 3 | 0.15 | 0.6981 | 3.02 | 0.0864 | 0.08 | 0.7817 |
| 4 | 3.17 | 0.0802 | 1.74 | 0.1921 | **5.90** | **0.0181** |
| 5 | **5.36** | **0.0266** | 1.21 | 0.2796 | 1.95 | 0.1717 |

## 3.2 Orientation

### 3.2.1 Nearest neighbour distance & horizontal orientation

There is large variation in the alignment between the reference fish and its nearest neighbour across crowding densities and over time (Figure 22) and there are no significant relationships (Mixed effect model, (a) Crowding: $F_{2,294}$=1.93, p=0.1475, (b) Day, $F_{2,294}$=0.07, p=0.9284, Appendix 9).

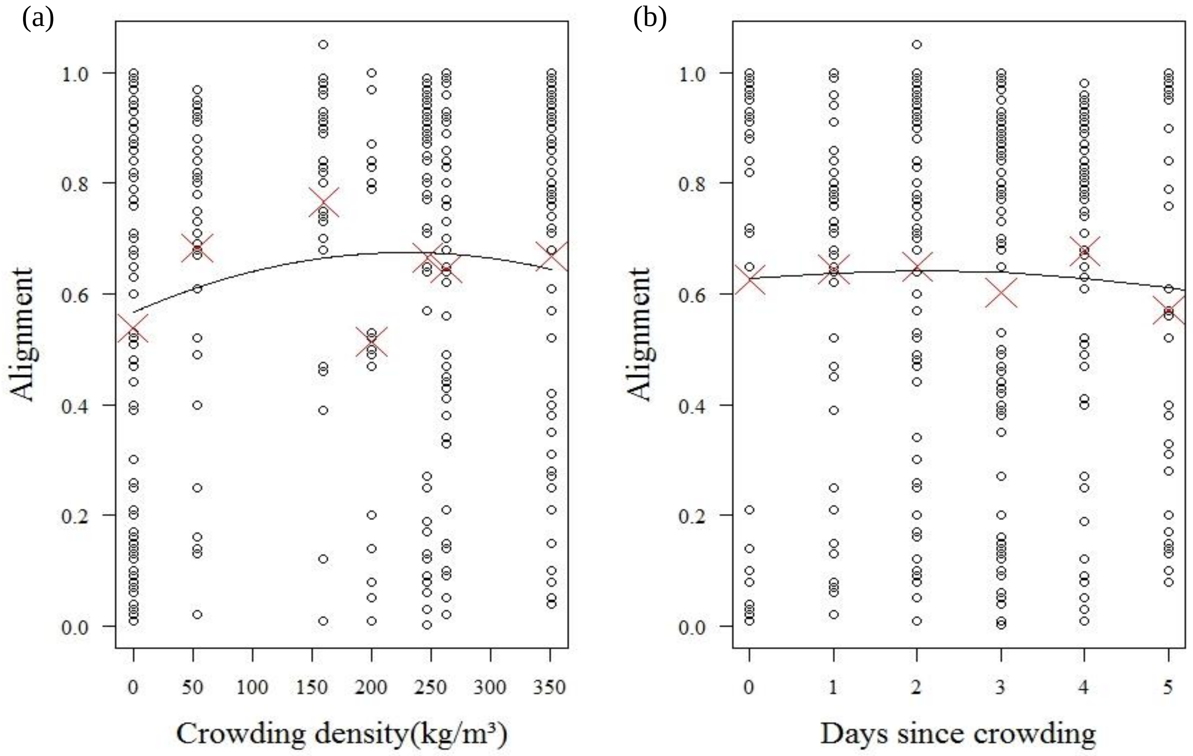


Figure 22: Plot showing the horizontal alignment between 10 reference fish each day and their nearest neighbour over (a) crowding densities (0-351 kg/$m^3$) and (b) 0-5 days since crowding. Red crosses show mean alignment.

Crowding density also has no significant effect on the relationship between NND and horizontal alignment (Linear mixed effects, $F_{1, 295}$=0.84, p=0.3590, Appendix 10) (Figure 23).

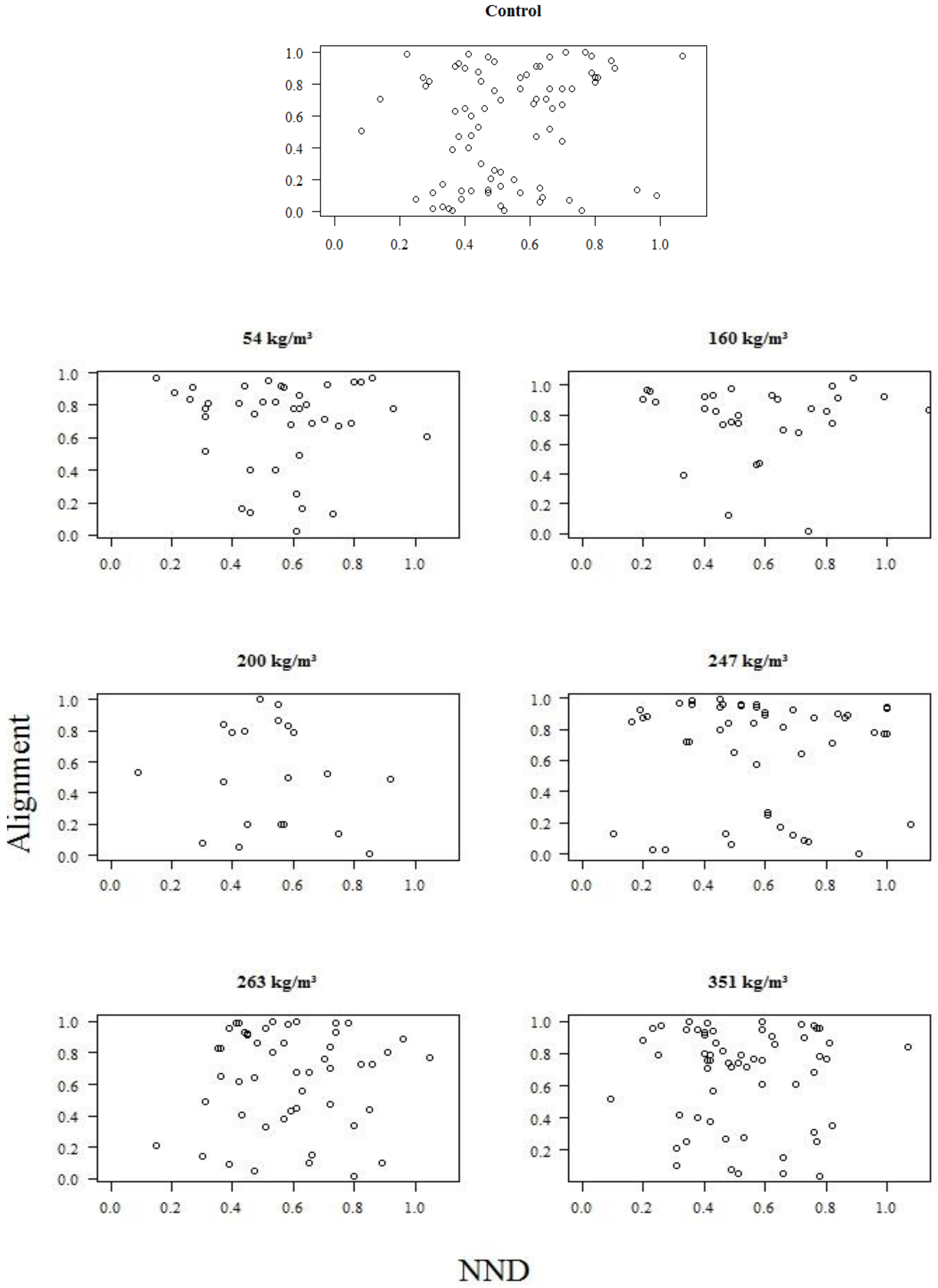


Figure 23: The relationship between horizontal alignment (0=oppositely aligned/180°, 1= perfectly aligned/0°) and the NND (Nearest Neighbour Distance): distance (BL) between 10 reference fish and their nearest neighbour in the control nets and crowding densities ranging from 54-351 kg/m$^3$ (all days combined).

There are also no significant relationships in NND across crowding densities and over time (Figure 24) (Mixed effect model (a) Crowding: $F_{2,294}$=1.66, p=0.1919, (b) Day, $F_{2,294}$=1.83, p=0.1629, Appendix 11).

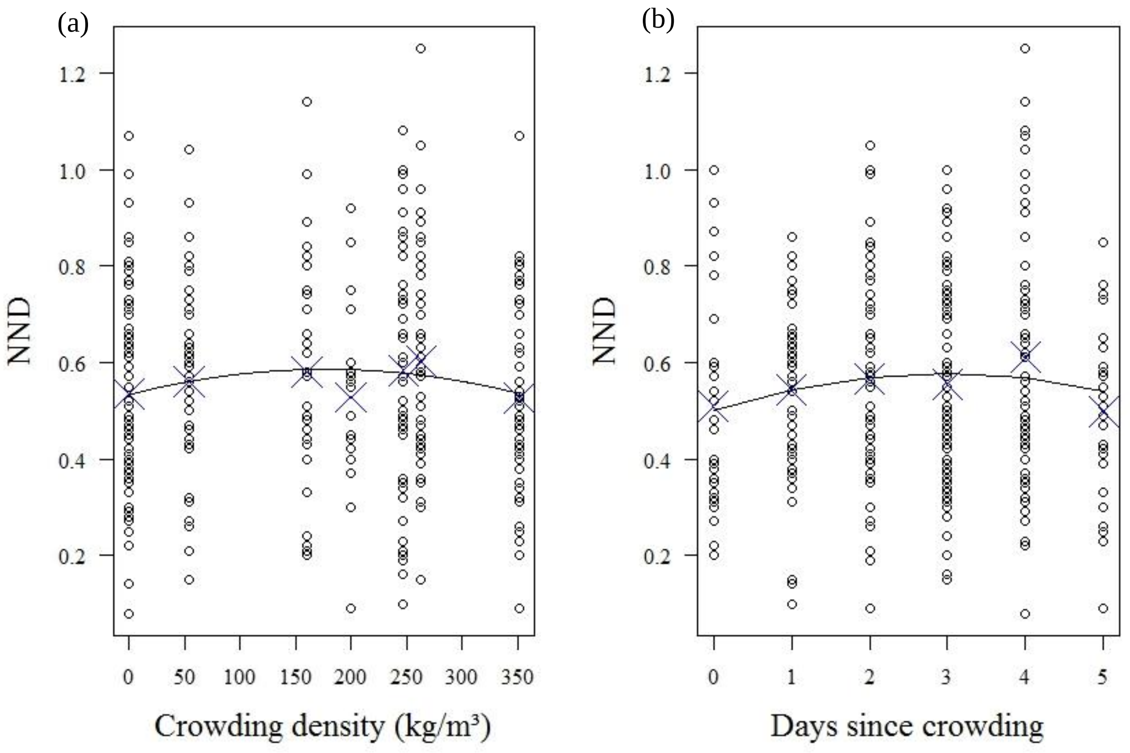


Figure 24: Plot showing the horizontal NND (BL) between 10 fish each day and their nearest neighbour over (a) crowding density (0-351 kg/$m^3$) and (b) 0-5 days since crowding. Blue crosses represent mean NND.

### 3.2.2 Vertical orientation

#### 3.2.2.1 Extreme orientation

After categorising the vertical orientation into extreme and normal orientation it was found that 83% (n=11078) of the fish were orientated normally with the remaining 13% (n=2219) orientated extremely.

Among the extremely orientated fish the overall preference was to be angled down (74%, n=1650) rather than up (26%, n=569) (Figure 25). There was no significant difference in preference between crowding densities (GLMM $F_{1,117}$=0.32, p=0.5730) or over time (GLMM $F_{1,117}$=0.25, p=0.6179, Appendix 12).

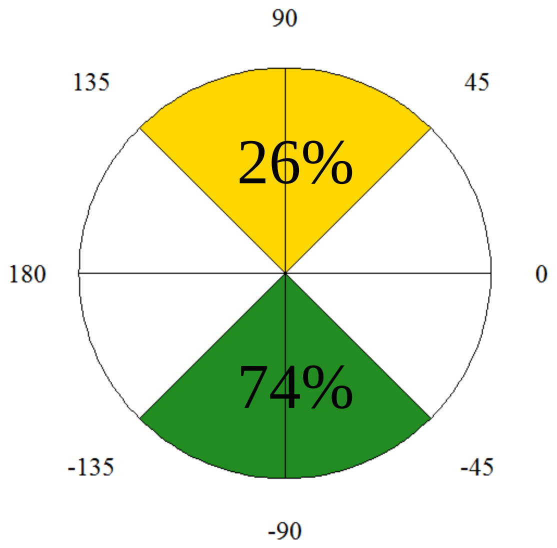


Figure 25: Fish orientated within 45-135° (yellow) are classified as extreme and up. The fish orientated within -45° and -135° are classified as extreme and down (green). Percentages show how the extreme fish were orientated.

The occurrence of extreme orientation was not affected by crowding density. However, extreme orientation significantly increased over the five days from <5% probability of occurrence at day 0 to a 20% probability at day 5 (GLMM, $F_{1,142}$=11.86, p=0.0008, Appendix 13) (Figure 26).

By comparison footage analysed of herring observed in the wild had only a 2% probability of extreme orientation (33 of 1445 measurements showed extreme orientations). This is lower than the probability at day 0 (Figure 26). Of those extremely orientated, 66% (n=22) were angled down and 33% (n=11) angled up.

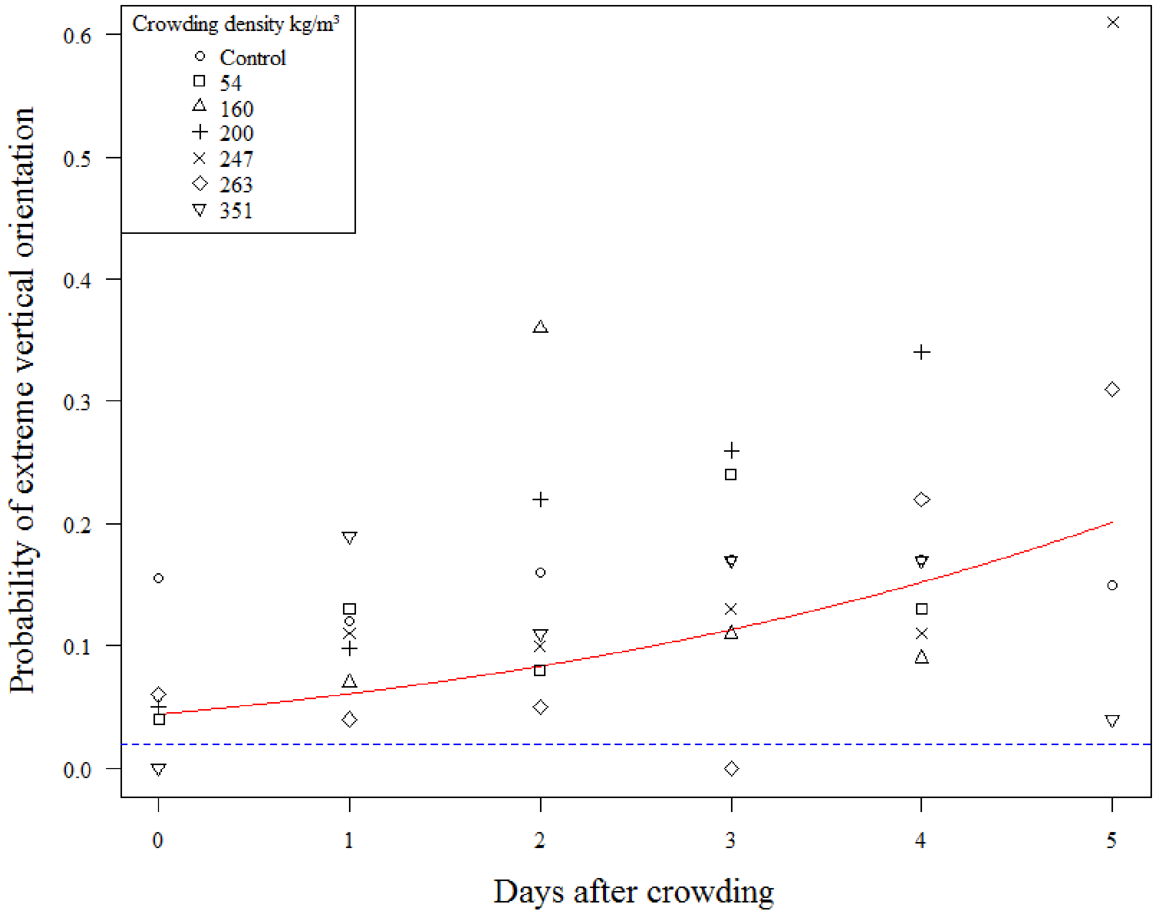


Figure 26: The relationship between the probability of extreme orientation (red predicted line from a Generalised Linear Mixed Model via PQL, $F_{1,142}$=11.86, p=0.0008) over days including raw data at all crowding densities (0-351kg/$m^3$). Blue dashed line shows probability of extreme orientation for the wild footage.

### *3.2.2.2 Variance*

There was no significant difference in variance in vertical orientation over time (Linear mixed effects, $F_{1,102}$=2.90, p=0.0918) or over crowding densities (Linear mixed effects, $F_{1,102}$=0.54, p=0.4634, Appendix 14) (Figure 27).

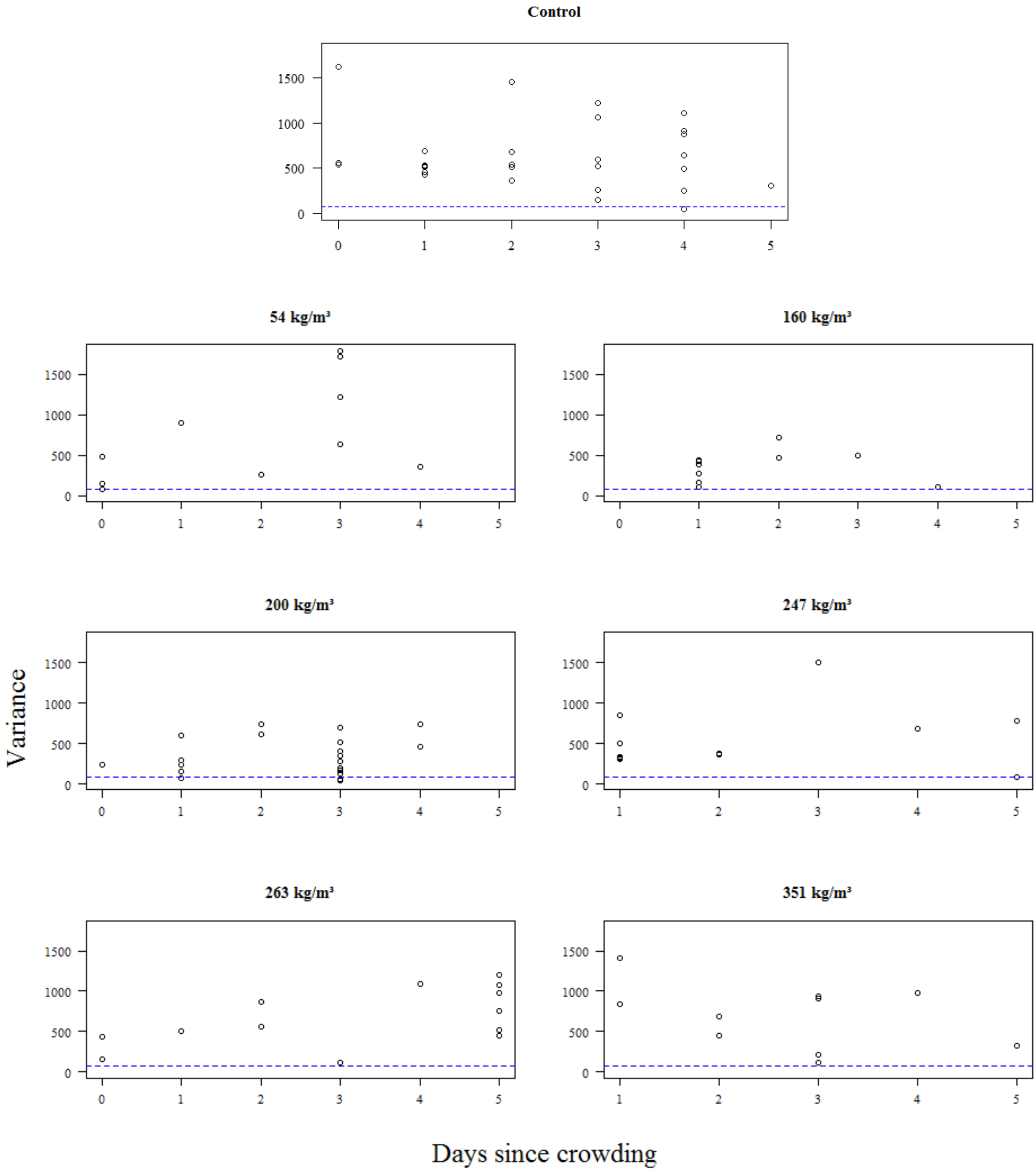


Figure 27: Variance in vertical orientation between 10 fish from the day of crowding (0) until day 4 or 5 in the control nets and over crowding densities ranging from 54-351 kg/$m^3$. The blue dashed line indicates the mean variance (78.9) for herring observed in the wild.

There was high variance in all the cages, with the highest mean variance of 759 at 54 kg/m$^3$ and the lowest of 347 at 200 kg/m$^3$. By comparison the herring observed in the wild showed a lower mean variance of 79 (Figure 27) with only 5 frames from the experimental cages showing variances equal or less than the wild. There was also a large range in variance in all the cages with the highest range found at 54 kg/m$^3$ (range=1722) and the lowest at 160 kg/m$^3$ (range=620). The wild herring have a lower range in variance of 232.

# 4. Discussion

From the two behaviour metric groups swimming activity and orientation investigated in this thesis, swimming activity has the most potential for use as a behavioural indicator of stress in fish following crowding. In particular the metric tail beat frequency, which showed a marked increase with crowding density. Tail beat frequency was also strongly correlated with mortality, suggesting it may be a strong indicator of stress. Interestingly only minor effects of crowding were observed for the other metrics investigated. This could be due in part to limitations in the data, but may also be indicative of captivity effects. Recommendations for future work will be discussed.

## 4.1 Data limitations

As the video footage used in this study was not recorded for the purpose of behavioural analysis, there were some limitations and difficulties faced. However the analysis methods were developed with these limitations in mind in order to minimise their impact and extract the most information possible from the data available.

The first limitation is that the behavioural observations made post-crowding were taken in a captive environment. This can create issues including: ensuring the volume of the cages is sufficient to not disrupt natural schooling behaviour, excessive changes in water movement could induce additionally stress, predators forming around the cages, and disturbing fish during monitoring of the cages (ICES, 2014). All of these can induce captivity stress. Therefore the confined environment of this study means the behavioural impairments and mortality observed may not be an accurate representation of those that would occur in a true fishery. The results are also based on two experiments, in one location, in specific conditions and at a specific time of year and therefore may not represent the behaviour of herring in commercial purse-seine fisheries generally. There were differences between the two experiments notably that the second experiment was towed, and as a result the fish were subjected to additional stress from towing and also an additional day in captivity. However, due to a low number of replicates (due to cost and time constraints) and the uneven crowding densities between the experiments, the two were combined for analysis. The data therefore serve to illustrate relative differences caused by time and crowding density (Marçalo *et al.*, 2013).

One limitation was the lack of systematic data. There was little vertical footage on some days, in some of the cages, in particular in the low crowding densities at days 0 and 1. These are the most critical days for determining whether behaviour can be used as an immediate indicator of stress. Additionally no video was collected on the termination day (day 5) for the first trial, due to poor weather conditions, meaning the data available for the lower crowding densities was reduced further. As the video camera was controlled manually and not fixed on the cage there was a large amount of camera movement. The sequence lengths were therefore reduced to enable individual fish to be tracked across frames. The angle of the video camera was also unknown. This meant that when video was sorted into vertical and horizontal footage, assumptions had to be made and angles that were not deemed to be approximately 90 or 180° to the surface were discarded, thus reducing the amount of usable footage. As the specific position of the camera within the cage was unknown the footage was split into two categories: footage shot midway down the cage and footage at the bottom of the cage.

More detailed limitations for each method will be discussed in the subsequent sections.

## 4.2 Swimming activity

### *4.2.1 Tail beat frequency*

Tail beat frequency significantly increased with increasing crowding density. This increase could reflect an adaptive stress response to increase the probability of survival in a suboptimal environment (Schreck *et al.*, 1997). At high crowding densities, such as those that occur during slipping, oxygen concentrations are significantly reduced creating hypoxic conditions (Dommasnes *et al*. 1994; Breen, *pers. comm.*). In these situations an increase in swimming activity can be advantageous: increasing the likelihood of finding more favourable conditions (higher $O_2$ concentrations) (Domenici *et al.*, 2000).

An increase in swimming speed at low oxygen concentrations has also been shown for herring (Herbert and Steffensen, 2006) and Atlantic mackerel exposed to thermal stress (Olla *et al.*, 1975). However the opposite has been seen in sardine, where crowded sardine displayed significantly lower swimming speeds than the control (Marçalo *et al.*, 2013). The explanation for this difference may be the warmer temperature at which sardine live and that sardines become exhausted faster than herring.

Scale loss can also be linked with swimming activity in herring (Olsen *et al.*, 2012). At a high crowding density the likelihood that fish collides with its neighbour increases. There is also a higher likelihood that fish on the outside of the school collide with the cage. As a result scale loss is expected to be higher. Elevated cortisol levels found in de-scaled fish indicate a stress response. Catecholamines are released prior to cortisol and therefore adrenaline (a catecholamine hormone) levels are elevated increasing a “fight or flight” response within the fish (Barton, 2002; Romero and Butler, 2007). This may be why there is an observed increase in swimming speed (Olsen *et al.*, 2012). Scale loss could therefore be one factor causing the increase in tail beat frequency shown in this study.

However an increase in swimming speed is only advantageous if the fish are able to find more favourable conditions. In the wild therefore an increase in swimming speed is an adaptive response increasing the fish’s survival likelihood, whereas in captivity the fish is not able to escape therefore a high swimming speed is maladaptive. Additionally, maintaining a high swimming speed requires high energy expenditure, thereby increasing the likelihood of exhaustion and a lethal stress response. In the present study the strong correlation between tail beat frequency and mortality suggests that increased swimming activity is a maladaptive stress response in captive herring. Over time tail beat frequency decreases at the highest crowding density (351kg/m$^3$). This could be due to fatigue, as these fish show the highest initial tail beat frequency at days 0 and 1. Physiologically this is supported by Olsen *et al.* (2012) who showed decreasing plasma glucose with increasing swimming speed, suggesting that herring died from glucose exhaustion. Tenningen *et al.* (2012) also found low glucose levels at high crowding densities with mortality rates up to 52%. The decrease in tail beat frequency could also be due to mortality. The fish that were most stressed at the start of the experiment may have died and therefore removed from the sample population. The metrics are then taken on the least stressed fish.

Towing is one potential bias that likely resulted in enforced swimming (Breen *et al.*, 2004). The fish caught for the second experiment, and subsequently exposed to the higher crowding densities, were towed from where they were caught to where the trials were conducted. Enforced swimming is known to cause stress in fish (Davis, 2007; Olla *et al.*, 1997) and therefore the final mortalities may have been elevated. This is supported by the higher mortality in the control group compared to the control in the first experiment. This may affect the behaviour of the fish, potentially exaggerating the effect crowding density had on tail beat frequency. However as the mortality in the control cage is significantly lower than in the trial cages, towing could not be the primary cause of mortality.

The length of each fish was assumed to be the mean length within each cage. This assumption creates some bias in the activity measures, as swimming speed is proportional to body length (He, 2010) and variation in tail beat amplitude increases with body length, but the relative differences between cages will remain the same. Previous studies have shown that fish of a smaller size and with a lower condition factor are more vulnerable to the effects of crowding (Tenningen *et al.*, 2012) and have a decreased survival rate after release or escape from fishing gear (Suuronen *et al.*, 1996; Marçalo *et al.*, 2010). Therefore as the experiments proceeded in this study, and the most vulnerable fish died, there was a higher likelihood that behavioural metrics are taken on larger fish, with a higher condition factor. This creates some bias when looking at changes in behaviour over time. Additionally, moribund fish have been shown to separate from the main fish group, moving either down towards the bottom of the cage (Mitchell *et al.*, 2002) or up towards the surface and the top of the cage (Vold *et al.*, 2014). If the former is true, then swimming activity, measured at the bottom of the cage, may represent fish whose behavioural deficits are greater.

Changes in behaviour have in fact been shown to be more species and stressor-specific indicators than plasma parameters (Xu *et al.*, 2006), which can lack concordance with mortality (Davis *et al.*, 2001). The strong correlation between tail beat frequency and mortality seen in this study suggests it is a sensitive index to not only crowding stress but also mortality within these experiments. Captivity could however have been the prime reason for why a high tail beat frequency is maladaptive in this study. Within a commercial purse-seine fishery, herring slipped at high densities may initially have an elevated swimming activity as a result of hypoxia, however if they swim to a more favourable environment post-crowding then recovery from hypoxic stress may be possible. Slipping exposes fish to more than one potentially fatal stressor, therefore delayed mortality may occur when increased swimming activity is combined with other factors, such as physical injury, causing deviations from behavioural norms to become too extreme to facilitate recovery.

### *4.2.2 Swimming modes*

Fatigued fish tend not to swim continuously (Riyanto *et al.*, 2014) and by switching from steady swimming to kick & glide the fish can save about 50% of its energy as gliding reduces the drag on the fish (Videler, 1996). Half tail beats also require less energy than full tail beats, due to reduced movement. It is suggested therefore that the swimming modes classified as “alternative” (“kick & glide” and half tail beat) are energy saving modes, indicative of stressful situations, whereas their counterparts, steady swimming and full tail beat, are more energy consuming although more effective for predator avoidance.

Given this, it would be expected that kick & glide and half tail beats would increase over time at high crowding densities, as the fish become fatigued from increased swimming activity. Although there was a significant difference between the occurrence of the modes kick & glide and steady swimming over crowding density, there was no consistent increase or decrease in either mode as crowding density increased. Kick & glide was common within all cages and all days with around half of the fish using it, which is not unexpected as herring are frequent kick & glide swimmers (Videler, 1996). Half tail beats were most frequent when the fish performed kick & glide and herring are thus combining energy saving swimming behaviours.

Half tail beats can also be due to captivity effects as a half tail beat can be used to facilitate turning. The occurrence of half tail beats and turning in the present study were therefore classified as alternative swimming modes and were expected to be high due to the circular nature of the cage. Turning greater than 90° was also seen in over half the fish analysed. This captivity effect could therefore have masked the effect of crowding. Due to the camera movement the likelihood of turning may also have been over or under estimated.

Overall the swimming modes measured gave a useful overview of how the fish were swimming within the cages. The mixture of swimming patterns observed within each cage suggests all the fish are not behaving the same. This breaks down one of the assumptions of schooling that fish are swimming in synchrony, suggesting depolarisation. This is further supported by the results from the orientation metrics. However as no significant changes were found with increasing crowding density it can be concluded that swimming modes are not good indicators of crowding stress.

### *4.2.3 Tail beat amplitude*

The tail beat amplitude was larger in the alternative swimming modes than in steady swimming modes. Tail beat amplitude, combined with tail beat frequency, determines the swimming speed of a fish. Mixtures of different swimming modes were observed within the cages. Therefore, in order for all the fish to swim at the same speed the different swimming modes must use different tail beat frequencies and amplitudes. For example, a fish using a half tail beat would have to either have a faster tail beat frequency or a larger tail beat amplitude than a fish swimming using a full tail beat, in order to travel at the same speed. Larger tail beat amplitudes are more energy efficient than faster tail beat frequencies (Liao *et al.,* 2003) making amplitude the most likely choice. This explains why the tail beat amplitude is higher in the energy efficient (alternative) swimming modes than in their counterparts.

The largest difference in tail beat amplitude between paired swimming modes was seen on the first day, as this may be when it is most important for all the fish to swim at the same speed. Immediately after being exposed to the stressor the fish may have a stronger schooling tendency, as falling away from the school would increase their likelihood of predation in the wild. This is supported by the observations of the high tail beat frequency and low extreme orientation. Over time, however, it is hypothesised that the fish become fatigued and therefore more fish may increase their tail beat amplitude and decrease their tail beat frequency in order to save energy. This could explain the smaller difference in tail beat amplitudes after the first day.

Notably the tail beat amplitudes measured in this study were exceptionally large. The tail beat amplitudes range from about 20-30% of body length; measured as a half tail beat. Videler (1996) stated that tail beat amplitudes usually ranged from 5-14% of body length, measured as a full tail beat. These figures are, however, based on a limited number of fish species, not including herring, in a situation with steady swimming and from controlled laboratory experiments. Among wild fish steady swimming is exceptional (Videler, 1996), especially with regards to dynamic schooling species such as herring. Because this study was carried out in the field, with further crowding and captivity stressors imposed on the fish, it is reasonable to assume a change in swimming and therefore higher tail beat amplitude compared to Videler.

Furthermore the different results could be due to the different methods used. In this present study tail beat amplitude was measured in two-dimensions, excluding the vertical orientation. When a fish was not orientated parallel to the camera’s field of view, estimates of its body length measurements could have been underestimated due to differences in the actual and apparent length of the subject. This in turn will overestimate tail beat amplitudes (see appendix 15). Although this did not appear to be a problem when carrying out data analysis, the results from vertical orientation do show fish exhibiting extreme orientation (≥45°) and therefore this could be an explanation for the large tail beat amplitudes found.

The larger tail beat amplitudes could be indicative of a general stress response; however they were seen across all cages, including the control, therefore not directly reflecting mortality. Tail beat amplitudes alone can thus not be used as an indicator of stress.

## 4.3 Nearest neighbour distance (NND)

A decrease in alignment over distance has been seen in previous studies as the transfer of information decreases with distance (van Olst and Hunter, 1970). However this was not seen in any of the cages in this study. It could be argued that as only one nearest neighbour was measured and as fish interact with several neighbours (Ballerini *et al.*, 2008), one nearest neighbour is too small to show a decrease. However then a strong alignment would be seen, but what was actually found was a range of alignments across all crowding densities. Captivity-induced stressors could also be the explanation for this, causing disorientation even at small distances between just one nearest neighbour. There was also no change in NND over crowding density or over time. This is contrary to the study by Marçalo *et al.* (2013), which showed an increase in NND in crowded fish compared to the control. One reason for the difference between studies could be the lack of synchronised orientations found in the controls in this study. A lack of synchrony suggests fish have a decreased awareness with their neighbours (Domenici *et al.*, 2000) and hence a larger NND.

## 4.4 Orientation

It is surprising that in this study both vertical and horizontal orientation were influenced so little by crowding. Depolarisation has been shown to occur as a result of stress, as stress can affect the sensory abilities herring require for schooling (Domenici *et al.*, 2000). Depolarisation was therefore expected to occur when crowding density reached a detrimental level. When fish show individual rather than collective behaviours, the organised structure within the school is lost; indicating the welfare of the fish has been compromised.

However in this study a high variance in vertical orientation and low alignment in horizontal orientation was seen across all cages, including the controls. The variance in vertical orientation was also higher than in the wild observations. This raises the question: why is the variance so large and the alignment so low in all the cages? Do the methods used not extract the information from the data? Or are the fish just showing no relationship in orientation? The wild footage validates the vertical orientation method, as it shows the expected result of a very low variance and a very low probability of extreme orientation; suggesting they are polarised. These fish were exposed to natural stressors, and an additional stressor of a diver filming the herring, which could be perceived as a predator. This suggests that, although there are limitations with the method, the caged herring are lacking uniformity in vertical orientation.

Polarised fish schools are dynamically stable and something needs to destroy this self-organization in order to cause depolarisation (Viscido *et al.*, 2004). Crowding does not appear to be the reason, but other stressors the fish are exposed to could be involved. Stressors are imposed on the fish at many stages of the experiment; pre- and during capture by the purse seine, for example noise-induced stress from the fishing vessels and during transfer from the purse seine to the cage. Perhaps the strongest stressors were within the cages: captivity-induced stress, predation from seabirds and disturbance during monitoring and camera movement. Although the size of the cages meant that the density of fish (control cages; 6.2-13.6 fish/m$^3$), was within the range of herring in the wild (Misund *et al.*, 1995; 0.03-22.2 fish/m$^3$), the captive environment and circular shape of the cage meant the fish had restricted movements whilst also being in an unfamiliar environment; these are captivity-induced stressors. However captivity stress doesn’t always result in a breakdown of synchronised orientation as has been shown in experiments with captive herring increasing their alignment in response to increased risk (Rieucau, *pers. comm.*). In this study captivity stress is not so prolonged that it results in death, as mortality rates were low in the control and low crowding densities, but cumulatively can be detrimentally stressful (Lockwood *et al.*, 1983; Wedemeyer, 1997) as indicated by the high variance in vertical orientation and low alignment in horizontal orientation.

One limitation with both orientation methods was that orientation was measured in two-dimensions and would be inaccurate if the fish is not swimming parallel with the camera. However, only fish that swam approximately parallel to the camera were selected in order to minimise this problem. Also measuring both horizontal and vertical orientation was also done in order to compensate for this limitation.

Another reason why there was no relationship between crowding density and orientation, and why there is such a large amount of variation seen in all the behavioural data, is that not all the fish will have had the same experiences. Each individual fish will have a different tolerance to crowding, based partly on their condition prior to capture but also due to the natural variation in individuals within a school (Martins *et al.*, 2012). The fish may also have different experiences during the crowding process based on their position within the school. Fish who were in the middle of the school during crowding will have experienced much lower oxygen concentrations than fish on the outside of the school, however fish on the outside of the school will have been more likely to be subjected to net abrasion (Tenningen *et al.*, 2012). Exposure to different stressors can cause different responses and therefore this can explain the variety of behaviours shown. With regards to orientation, if individual fish are showing different swimming modes and levels of activity to their neighbours, then it is hard for the fish to remain swimming in synchrony and fish have to adjust their orientation and swim in a way to prevent collisions. Despite no observed synchrony, the fish are trapped in a collective pattern due to the captive environment and have to respond to the behaviour of others (Martins *et al.*, 2012). If therefore the fish were observed in the wild, stronger effects of crowding may have been seen.

### *4.4.1 Extreme vertical orientation*

An interesting observation was the increase in extreme vertical orientation over time. One possible explanation is that over time a seabird population gathered around the cages. These could have presented a predatory threat to the herring, as the cages floated on the surface and were not covered. This could be linked to an increase in extreme vertical orientation, as three quarters of the extreme orientations represented diving rather than rising. The herring could therefore be diving in order to avoid seabird predation, and the increase in extreme orientation was a response to the increase in predation risk. Diving has also been observed within captive herring encountering a simulated predator (Rieucau *et al.*, 2014) and within the wild as a response to being predated upon by saithe (Pitcher *et al.*, 1996), puffins (*Fratercula arctica;* Axelson *et al.*, 2001*)* and killer whales (*Orcinus arca;* Nøttestad and Axelson, 1999).

Captivity-induced stress provides another explanation. On the day of crowding, extreme vertical orientation was seldom observed. Having just been exposed to a new captive environment, an individual may try to stay as “normal” as possible. If it deviates from “normal” (extreme vertical orientation) it will stand out from the rest of the school which may increase its likelihood of predation in the wild. This can also be linked to the swimming activity results. The day of crowding showed the highest swimming activity and lowest probability of extreme orientation, which are both anti-predator responses. This is supported by Viscido *et al.* (2004) who showed polarized fish groups were faster than non-polarized groups. Over time however the herring adapt to their captive environment and more focus can then be put on external stimuli. Each day the fish are monitored directly at the surface of the cages, which could disturb the fish. Over time as the fish become more aware of this external stimulus the fish dive in a startle response to the disturbance and could therefore provide another explanation for why there is an increase in diving over time. This could also be the reason why diving was more common than rising in the wild fish as the fish may dive in response to the diver.

## 4.5 Recommendations for future work

This study used data from experiments where fish were stored in cages, after crowding. However commercial fisheries need information before and during crowding within the purse seine. Further experiments may still use cages and therefore first recommendations shall be made to improve video collection, with particular reference to caged experiments. After this recommendations shall be made for how the proposed metrics could be used as an early warning indicator within the purse seine.

### *4.5.1 Video collection*

In order to improve the experimental design more systematic video footage should be taken, each day, in each of the cages and for a longer period of time; providing more balanced data for analysis. As the highest mortalities are expected in the first 24-48 hours after crowding (Huse and Vold, 2010; Suuronen *et al.*, 1996), and this is when the greatest behavioural differences were seen in this study, more frequent observations should be made during this time. In reality the cages were monitored every 24 hours as a balance between disturbance, resources required and data generation (ICES, 2014).

Swimming activity was the most promising behavioural metric and as swimming movements are often made laterally in the horizontal plane, vertical footage contains the most information (Videler, 1996), future studies should therefore collect vertical video footage. In this study the vertical footage was taken from the bottom of the school. The camera was positioned towards the surface and therefore the daylight provided a good contrast with the fish, aiding analysis. This is preferred to vertical footage taken from above. A video camera in a fixed position, at the bottom of the cage, throughout the experiment that could be activated and deactivated from the surface would reduce the disturbance whilst allowing more frequent video collection.

The lack of a third dimension in the images was one of the most restricting limitations in this thesis, therefore stereoscopic cameras would also be extremely beneficial. The density of the fish school could then be directly measured by counting the number of fish per $m^3$. This would allow accurate measurements of NND, as well as orientation, building up a 3D image of the school. Knowing the exact position of the camera in relation to the surface and the cage would also allow depth measurements to be made and ensure footage used was at 90° to the surface. Stereoscopic cameras and tilt measurements could be used to achieve this. Ideally the camera should be in a fixed position to reduce the amount of movement in the video footage which would allow an individual's behaviour to be tracked over a longer time period. Acoustic techniques would also compliment the video footage and would provide another, indirect, measure of fish density within the cages from which mean NND could be inferred.

Physiological data from the 2012 experiments is lacking. Whilst comparisons have been made with physiological data collected from similar experiments conducted in 2008, 2009 (Tenningen *et al.*, 2012; Olsen *et al.*, 2012) it would be useful to have the physiological data from 2012 to see if behaviour, in particular tail beat frequency, corresponds to cortisol, lactate and glucose levels, thus linking physiology, behaviour and mortality.

The collection of behavioural metrics in this study was very time consuming and done manually. In order for behaviour to be used as an immediate indicator of stress, and hence useful in the commercial purse-seine fishery, this needs to become more automated. There have been difficulties in automated systems within high fish densities and whilst developments have been made in recent years (Miller and Gerlai, 2007; Stien *et al.*, 2007; Xu *et al.*, 2006) a real-time image-processing method still needs to be developed and the processing speed increased in order to be used commercially.

### *4.5.2 Can tail beat frequency be used as an early warning indicator?*

Sampling during the capture process and early crowding would be extremely beneficial, as this is the stage at which fishermen decide the outcome of the catch. A small amount of video was taken during late crowding but the quality was poor due to the high densities of fish resulting in loss of light. Sampling during the late stages of crowding is likely to be too late to minimise mortality. Tail beat frequency could therefore be analysed during the early stages of crowding in the low and intermediate crowding densities, where visibility should be greater. The resolution of footage during crowding could also be improved through the use of artificial light, e.g. far-red light. Tail beat frequency could be counted quickly either through a rough visual count or by analysing just a few individuals.

In order to determine the baseline for tail beat frequency, analysis would have to be done on the tail beat frequency pre-crowding. Using this as the control, the tail beat frequency could then be measured during crowding and if the tail beat frequency has increased above a predetermined threshold (20% in this study) then mortality is likely to be high. In that case the catch has to be kept, as in Norway it is illegal to release dead or dying fish (Fiskeridirektoratet, 2004). If the tail beat frequency is below the threshold, it suggests mortality may be low and the fish can be slipped. However for this to be used in practice the relationship between tail beat frequency and mortality would need to be tested more rigorously during crowding in order to determine the threshold at which mortality is likely to occur. As the tail beat frequency was also measured post-crowding in this study, the tail beat frequency during crowding may be very different. Comparisons between tail beat frequency pre, during and post-crowding would also be useful to analyse any captivity effects. If the increase in tail beat frequency found in this study is predominately a captivity effect then it would not be a good welfare indicator within the purse-seine fishery.

If tail beat can be used as indicator of welfare it may also have the potential for determining the quality of the fish, as quality is strongly linked to welfare (Ellis *et al.*, 2002). This is also supported by previous studies which have found larger more robust fish have a higher condition factor (Marçalo *et al.*, 2010) and a lower tail beat frequency (Videler and Wardle, 1991) than smaller fish. Therefore if the quality of the fish could be determined pre-crowding, in some cases, crowding may not be needed, as determining the quality is one of the fishermen’s incentives for crowding.

Whilst ideas have been discussed for using tail beat frequency as an immediate indicator of stress, the practicalities for using it within the purse-seine fishery have many limitations. One idea would be to combine tail beat frequency with vitality assessments. Out of all the metrics analysed in this study only tail beat frequency seems to have any potential for use as an indicator of crowding stress. This suggests that the more complex and general behavioural metrics may not be the best indicators of stress and mortality. Current research is focusing on the most basic reflex behaviours to evaluate the status of the animal: the vitality. RAMP (Reflex Action Mortality Predictor) vitality assessments are one such technique that tests the presence or absence of a combination of pre-determined reflexes on an individual fish over a representative range of stressors (Davis, 2010). This method has been shown to correlate well with mortality in a range of species (Davis, 2010; Raby *et al.*, 2012; Humborstad *et al.*, 2009). As tail beat frequency showed a strong correlation with mortality in this study, it could also be a good additional vitality measure for herring in combination with free swimming reflexes. Future studies could also look at potential reflexes that could be obtained from video footage if the recommendations outlined are made.

## 4.6 Concluding remarks

From the behavioural metrics measured: tail beat frequency, amplitude, swimming mode, vertical and horizontal orientation, and nearest neighbour distance; only one has the potential to be used as an indicator of stress – tail beat frequency. Changes in activity have been observed to be a sensitive indicator of stress (Schreck *et al.*, 1997) and therefore it is not unexpected that this was the best indicator of stress in this study, however it is disappointing that this was the only metric to be affected by crowding. The fish were exposed to many stressors in this study, in addition to the crowding stress. Although crowding stress appears to drive mortality, the cumulative effect of the other stressors, in particular captivity stress, appear to strongly affect herring's behaviour thereby masking the effect of crowding. The limitations in the data could also explain why most of the metrics appear to be unaffected by crowding.

However, despite these limitations swimming activity was shown to increase with crowding, which may lead to exhaustion and high mortalities at the higher crowding densities. Tail beat frequency is therefore the best indicator of stress from the metrics measured. As a preliminary study it can be concluded that tail beat frequency has the potential for being a useful indicator of mortality. This metric should be tested more rigorously pre-crowding and during crowding, while making the recommended improvements to video collection in order to analyse the effect of captivity. This will determine the potential tail beat frequency has as a welfare indicator during purse-seining.

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# Appendices

# Appendix 1

Days in which vertical and horizontal video footage was available and the behavioural metrics which correspond to both camera angles.

| Crowding density kg/m$^3$ | Days since crowding | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** | **5** |
| **Control-1** | | ✔ | ✔ | ✔✓ | ✔✓ | |
| **Control-2** | ✔✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ |
| **54** | ✔ | ✔✓ | ✔✓ | ✔✓ | ✔✓ | |
| **160** | | | ✔✓ | ✔✓ | ✔✓ | |
| **200** | ✔ | | ✔✓ | ✔✓ | ✔ | |
| **247** | ✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ |
| **263** | ✔✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ |
| **351** | ✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ | ✔✓ |

✓**=Vertical footage**

✔=**Horizontal footage**

## Metrics

- Swimming activity
-Horizontal Orientation

- Position change
- Vertical Orientation

# Model outputs

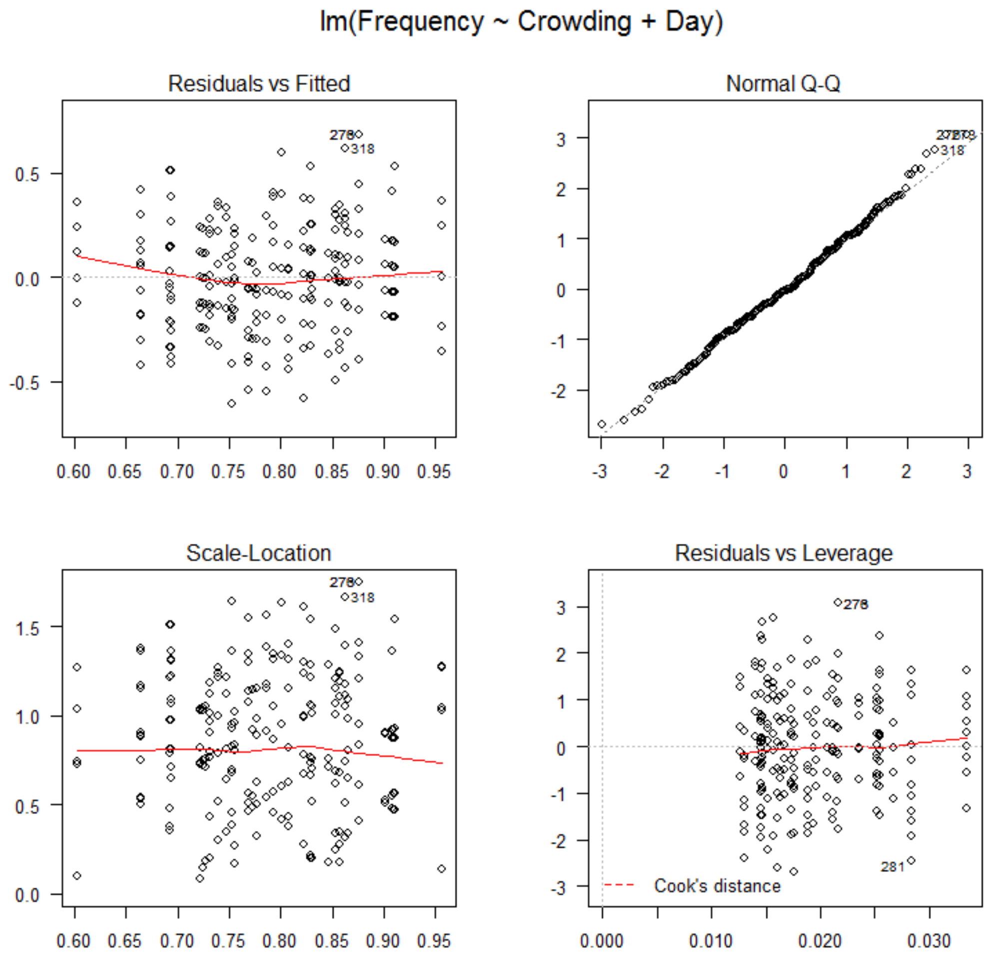
## Tail beat frequency

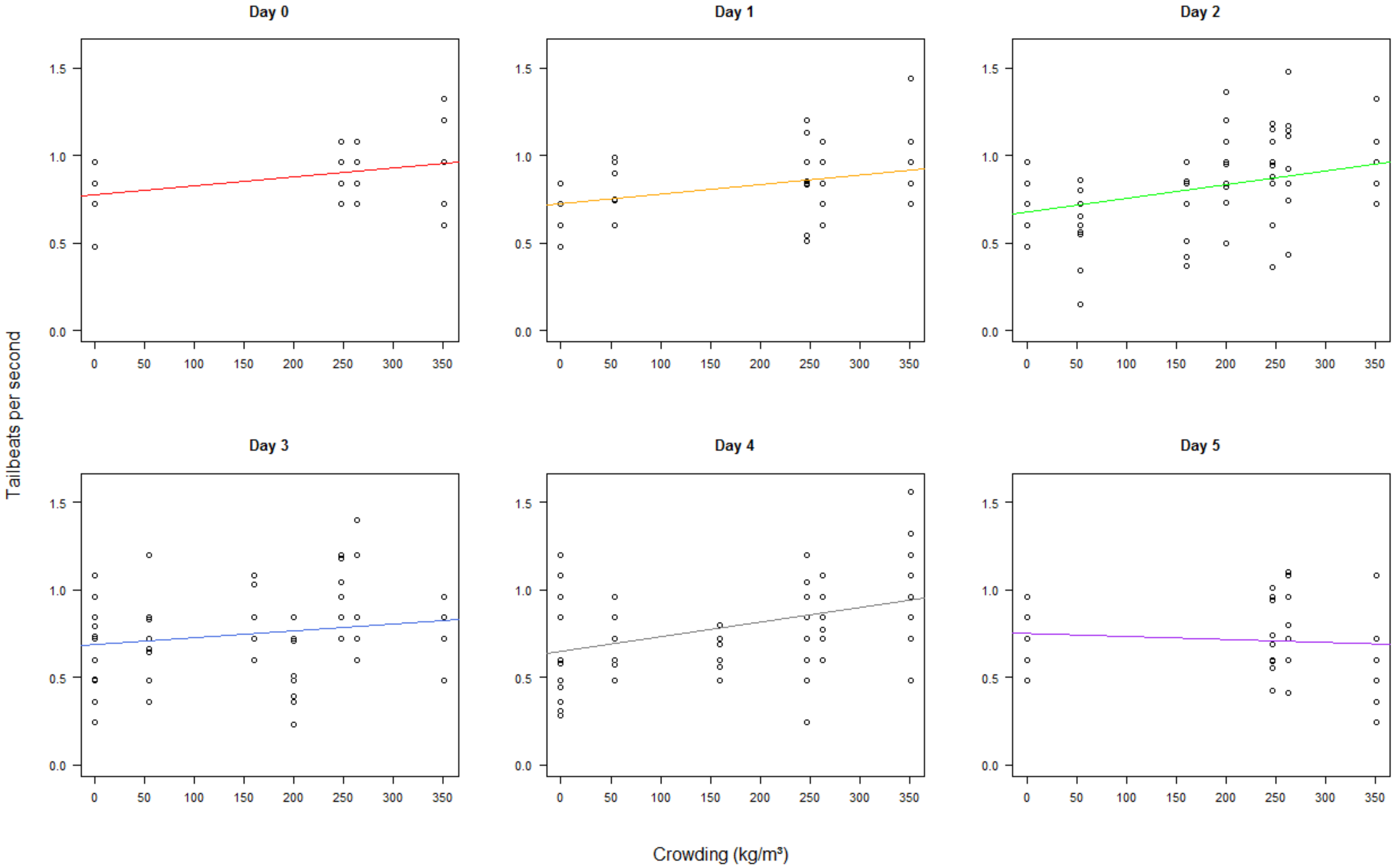
## Appendix 2

Model summary: lm(Frequency~Crowding+Day)

| *Day0 | Estimate | Standard Error | *t*-value | *p*-value |
|---|---|---|---|---|
| (Intercept) | 7.73x10$^{-1}$ | 4.12x10$^{-2}$ | 18.75 | <.0001 |
| Crowding | 5.23x10$^{-4}$ | 9.63x10$^{-5}$ | 5.42 | **<.0001** |
| Day1 | -4.46x10$^{-2}$ | 4.81x10$^{-2}$ | -0.93 | 0.3540 |
| Day2 | -4.78x10$^{-2}$ | 4.49x10$^{-2}$ | -1.07 | 0.2874 |
| Day3 | -1.09x10$^{-1}$ | 4.40x10$^{-2}$ | -2.47 | **0.0140** |
| Day4 | -7.93x10$^{-2}$ | 4.52x10$^{-2}$ | -1.76 | 0.0801 |
| Day5 | -1.70x10$^{-1}$ | 5.04x10$^{-2}$ | -3.38 | **0.0008** |

Adjusted $R^2$:0.11, *F*-statistic: 7.794 on 6 and 339 d.f. , *p*-value=<.0001





The relationship between tail beat frequency and crowding density (0-351kg/$m^3$) over 6 days (0-5 days after crowding). The predicted lines are from the linear model: lm(Frequency~Crowding) $R^2$=0.11, ANOVA, $F_{1,344}$=30.58, p<0.0001

ANCOVA values showing the effect crowding and day have on tail beat frequency

| | Sum of squares | Mean square | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|---|---|
| Crowding | 1.5972 | 1.5972 | 31.4882 | 1, 339 | **<.0001** |
| Day | 0.7748 | 0.1550 | 3.0551 | 5, 339 | **0.0103** |

## Appendix 3

### Start and End

One-way ANOVA values showing whether there is a difference in tail beat frequency between the start and end of the experiment.

| | Sum of squares | Mean square | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|---|---|
| Daygrouped | 0.67 | 0.67 | 12.28 | 1, 344 | **0.0005** |

## Appendix 4

### ANOVA values showing tail beat frequency for each crowding density over day

| Crowding (kg/m$^3$) | Sum of squares | Mean square | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|---|---|
| 0 | 0.1063 | 0.0212 | 0.5116 | 5, 74 | 0.7666 |
| 54 | 0.2336 | 0.0779 | 2.0091 | 3, 34 | 0.1312 |
| 160 | 0.0819 | 0.0410 | 1.5133 | 2,26 | 0.2390 |
| 200 | 0.8233 | 0.8233 | 16.152 | 1,17 | **0.0009** |
| 247 | 0.4634 | 0.0927 | 1.918 | 5, 54 | 0.1064 |
| 263 | 0.1016 | 0.0203 | 0.463 | 5,54 | 0.8020 |
| 351 | 1.6140 | 0.3328 | 5.54 | 5,54 | **0.0003** |

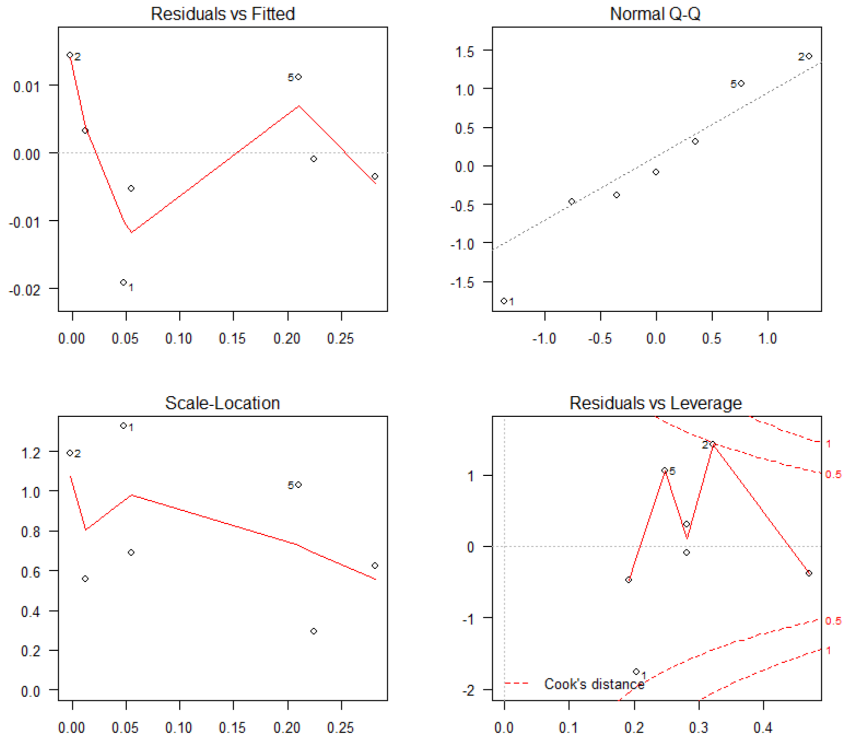
# Appendix 5

## Mortality & Tail beat frequency

Model summary: lm(Mortality~Frequency)

| *Mortality | Estimate | Standard Error | *t*-value | *p*-value |
|---|---|---|---|---|
| (Intercept) | -0.98 | 0.05 | -20.62 | <.0001 |
| Tail beat frequency | -0.09 | 0.03 | -3.50 | **<.0001** |

Adjusted $R^2$:0.99, *F*-statistic: 540 on 1 and 5 d.f. , *p*-value=$2.75 \times 10^{-6}$



# Appendix 6

## Swimming modes

### Kick & glide/steady swimming

Model summary: GLM (Kick & glide ~ Crowding, family=binomial)

| *Kick & Glide | Estimate | Standard Error | *z*-value | *p*-value |
|---|---|---|---|---|
| (Intercept) | 0.02 | 0.20 | 0.08 | 0.9372 |
| Crowding | $-1.9 \times 10^{-3}$ | $9.1 \times 10^{-4}$ | -2.1 | **0.0349** |

AIC=444.61, Null deviance: 445 on 327 d.f., Residual deviance: 441 on 326 d.f.

# Appendix 7

## Tail beat amplitude

Model summary:

lme (Tail beat amplitude ~ Crowding, random =~+1|Sequence)
lme (Tail beat amplitude ~ Day, random=~+1|Sequence)

| *Tail beat amplitude | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 2014.94 | 1,311 | <.0001 |
| Crowding | 0.0198 | 1,33 | 0.8889 |
| | | | |
| (Intercept) | 1838.34 | 1,311 | <.0010 |
| Day | 0.2282 | 5,29 | 0.9472 |

# Appendix 8

Tail beat amplitudes for swimming modes over days – maximum likelihood analysis

## Gait

lme (Tail beat amplitude[Day==0] ~ Gait[Day==0], random =~+1|Sequence)*repeated for all days

| Day | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| 0 | 1.9087 | 1,35 | 0.1761 |
| 1 | 0.2014 | 1,43 | 0.6559 |
| 2 | 0.0007 | 1,61 | 0.9791 |
| 3 | 0.1517 | 1,70 | 0.6981 |
| 4 | 3.1652 | 1,61 | 0.0802 |
| 5 | 5.3569 | 1,35 | **0.0266** |

## Turning

lme (Tail beat amplitude[Day==0] ~ Turning[Day==0], random =~+1|Sequence)$^{\text{*repeated for all days}}$

| Day | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| 0 | 9.5947 | 1,35 | **0.0038** |
| 1 | 9.7644 | 1,43 | **0.0032** |
| 2 | 0.0922 | 1,61 | 0.7624 |
| 3 | 3.0189 | 1,70 | 0.0867 |
| 4 | 1.7399 | 1,61 | 0.1921 |
| 5 | 1.2063 | 1,35 | 0.2796 |

## Tail beat

lme (Tail beat amplitude[Day==0] ~ Tail beat[Day==0], random =~+1|Sequence)$^{\text{*repeated for all days}}$

| Day | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| 0 | 6.6710 | 1,35 | **0.0141** |
| 1 | 12.0061 | 1,43 | **0.0012** |
| 2 | 0.2813 | 1,61 | 0.5978 |
| 3 | 0.0774 | 1,70 | 0.7817 |
| 4 | 5.9043 | 1,61 | **0.0181** |
| 5 | 1.947 | 1,35 | 0.1717 |

## Appendix 9

### Day & Crowding on Horizontal Alignment

lme(Alignment~poly(Day,2),random=~+1|Sequence)

lme(Alignment~poly(Crowding,2),random=~+1|Sequence)

| *Alignmnet | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 839.88 | 1,294 | <.0001 |
| Crowding | 1.93 | 2,294 | 0.1475 |
| | | | |
| (Intercept) | 747.90 | 1,294 | <.0001 |
| Day | 0.07 | 2,294 | 0.9284 |

## Appendix 10

### NND and Horizontal alignment

lme(Alignment~Distance,random=~+1|Sequence)

| *Distance | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 2290.30 | 1,295 | <.0001 |
| Alignment | 0.84 | 1,295 | 0.3590 |

## Appendix 11

## Day & Crowding on NND

lme(NND~poly(Day,2),random=~+1|Sequence)

lme(NND~poly(Crowding,2),random=~+1|Sequence)

| *Alignmnet | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 2300.62 | 1,294 | <.0001 |
| Crowding | 1.66 | 2,294 | 0.1919 |
| | | | |
| (Intercept) | 2302.95 | 1,294 | <.0001 |
| Day | 1.83 | 2,294 | 0.1629 |

## Appendix 12

## Vertical orientation

## Extreme - Preference up or down

glmmPQL(Up_Down~Day, random=~+1|FishgroupID, family=binomial,
glmmPQL(Up_Down~Crowding_density, random=~+1|FishgroupID, family=binomial

| *Extreme angle | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 15.4199 | 1,2100 | 0.0001 |
| Day | 0.2502 | 1,117 | 0.6179 |
| (Intercept) | 15.4026 | 1,2100 | 0.0001 |
| Crowding_density | 0.3196 | 1,117 | 0.5730 |

## Appendix 13

### Extreme - Day & Crowding density

Day value from:

glmmPQL(Extreme_angle~Day, random=~+1|FishgroupID, family=binomial

Crowding value from:

glmmPQL(Extreme_angle~Day+Crowding, random=~+1|FishgroupID, family=binomial

| *Extreme_angle | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 238.51 | 1,13153 | <.0001 |
| Day | 11.8562 | 1,142 | **0.0008** |
| | | | |
| Crowding_density | 0.0949 | 1,141 | 0.7585 |

## Appendix 14

### Variance in vertical orientation – Crowding density & Day

Model summary:

lme (Variance ~ Crowding, random =~+1|Sequence)
lme (Variance ~ Day, random=~+1|Sequence)

| *Variance | Value of *F*-statistic | d.f. | *p*-value |
|---|---|---|---|
| (Intercept) | 218.6495 | 1,102 | <.0001 |
| Crowding | 0.5417 | 1,102 | 0.4634 |
| | | | |
| (Intercept) | 223.6698 | 1,102 | <.0010 |
| Day | 2.8961 | 1,102 | 0.0918 |

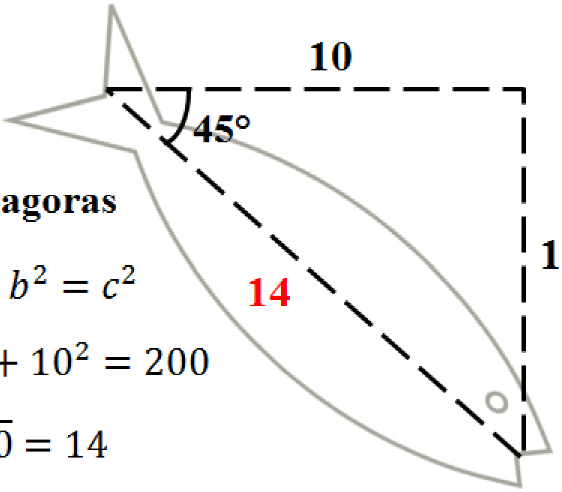
## Appendix 15 – Biases in tail beat Amplitude

**Pythagoras**

$$a^2 + b^2 = c^2$$

$$10^2 + 10^2 = 200$$

$$\sqrt{200} = 14$$



If tail beat amplitude was measured as 20% of the body length then:

**Measured amplitude**:
10 x 0.20 = 2

**Actual %**:
2/14 = 0.14 = **14%**

If, for example, a fish was vertically orientated at 45°, its apparent length would be 10 compared to its actual length of 14. In this case, the measured tail beat amplitude was 20% of body length, but when corrected for actual length then the actual tail beat amplitude is 14%. If the fish was orientated more than 45% then the actual tail beat amplitude would be further reduced.