Assessing mackerel behaviour following crowding-induced stress in purse seine fisheries

Thesis in partial fulfilment of the degree

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Kirsten Howarth

Department of Biology

University of Bergen

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"So long, and thanks for all the fish."

-- Douglas Adams

ABSTRACT

The practice of slipping in purse seine fisheries has been shown to cause high levels of delayed mortality in released fish. This unaccounted mortality could lead to bias in stock assessments, and brings the sustainability of these fisheries into question.

Behavioural stress responses of individual mackerel (*Scomber scombrus* L.) and mackerel schools were analysed using visual and acoustic methods under non-lethal crowding and hypoxic conditions in purse seine simulations. Metrics observed included tail beat frequency and amplitude, and school vertical distribution and density. Tail beat frequency and school density were the best potential stress indicators for welfare in mackerel during purse seine fisheries – with significant increases in tail beat frequencies and densities of up to 60 fish m^{-3} with crowding, as well as evidence of adaption and recovery over treatment time. The addition of hypoxia shows an interaction of effects on these metrics, showing no additive effect to the crowding treatment, and suggests a behavioural trade-off in mackerel between the maintenance of school structure and oxidative stress. Further study into the sole effect of hypoxia on mackerel behaviour is recommended.

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1. INTRODUCTION

In purse seine fisheries, the release of unwanted catch from the purse seine net – known as 'slipping' – has been shown to cause high levels of fish mortality (Lockwood et al., 1983; Huse & Vold, 2010; Tenningen et al., 2012a; Marçalo et al., 2013; Arregi et al., 2014). Stressors including high crowding densities and hypoxia are present within the purse seine during the capture process (Davis, 2002; Olsen et al., 2012; Tenningen et al. 2012b). These may cause unaccounted mortality of fish, either due to physiological reasons such as skin damage (Lockwood et al., 1983; Bellido et al., 2011) and exhaustion (He, 1993; Domenici et al., 2000; Broadhurst, 2006), or behaviourally-induced reasons, such as increased vulnerability to predators upon release (Broadhurst, 1998; Ryer, 2002; Ryer, 2004; Zhou et al., 2007). This unaccounted mortality could lead to bias in stock assessments (Mesnil, 1996; Breen & Cook, 2002), and brings the sustainability of the fisheries into question. Studying the behaviour of fish post-slipping could provide behavioural indicators of stress in fish, and help to provide thresholds for safe release from a purse seine (Huntingford et al., 2006).

1.1. PURSE SEINE FISHERIES

Purse seining accounts for approximately 30% of the global catch of fish every year, making it the most productive of fishing techniques (Watson et al. 2006). Purse seine fisheries mainly target pelagic schooling species all over the world, such as mackerel, herring, tuna and blue whiting. Purse seining is a non-selective, but highly efficient method of fishing (Ben-Yami, 1994), surrounding and capturing an entire school of fish with one large seine net, with an upper line (head-rope) attached to floats ensuring the net remains on the surface. Rings along the bottom edge of the net, through which a cable (the purse-line) extends, allow the fishermen to fully enclose the fish, preventing them from escaping downwards. After closure of the net by hauling in the purse-line, the net is slowly hauled aboard (Figure 1). As space within the net is gradually reduced, the captured fish become more densely packed and are then taken aboard either by hauling the net on-board, or bringing it alongside the vessel and using a pumping system (Lockwood et al., 1983; Ben-Yami, 1994). Depending on the fishery, a purse seine net can be several kilometres long and more than 200m deep. As a fishing technique, it is also regarded as economical due to its fuel-efficiency; by targeting schooling fish species, low fuel consumption still yields high catches, with approximately 0.1L of fuel per kilogram of catch (Suuronen et al., 2012).

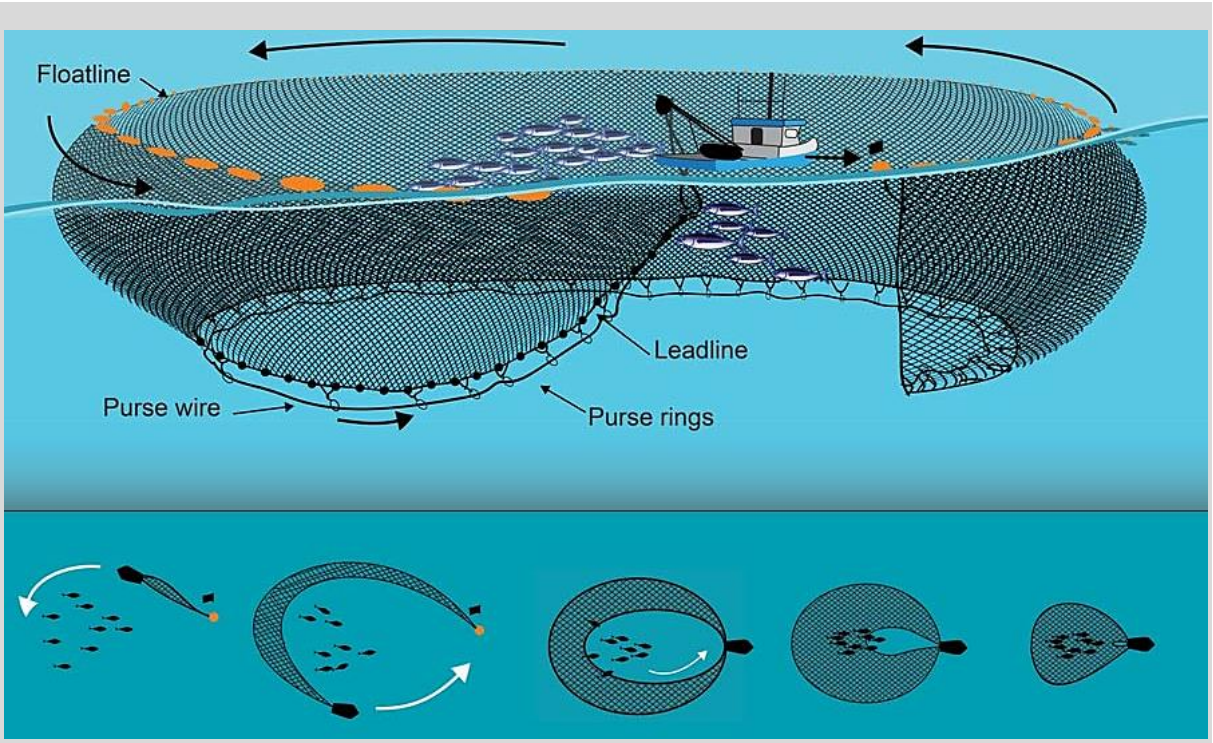

Figure 1. Anatomy of a purse seine and the process of setting the net.
Source: Australian Fisheries Management Authority.

1.2. SLIPPING

Slipping refers to a practice whereby fish caught in a net – typically a purse seine – are subsequently "released into the sea without being brought onboard a vessel" (Kelleher, 2005). Slipping can occur for a number of reasons – for example, if the catch is too large for the boat capacity or quota, of the wrong or non-target species, or due to high grading whereby less valuable species or size-classes are discarded to leave space for more valuable catch (Bellido et al., 2011). Discards are defined as the portion of a catch of fish which is not retained on board during commercial operations and is returned, often dead or dying, to the sea (Catchpole et al., 2005). Discarded bycatch has been estimated at approximately 8% of the worldwide fisheries catch (Kelleher, 2005). Slipping differs slightly in that fish are released from a purse seine net prior to being brought onboard. However, the exclusion of slipping mortality can lead to variations in standard assessment models (Stefansson, 2003).

Slipping induces high mortality rates in released fish following high crowding densities within a purse seine, and as no data is collected on frequency of slipping events, this may lead to underestimation of fishing mortality in purse seine fisheries (Huse & Vold, 2010; Breen et al. 2012). Slipping mortality may be caused by physical damage of the fish from contact with the gear and other fish during crowding, but has also shown to be dependent on crowding time and

density (Tenningen et al. 2012a, Marçalo, 2013; Arregi et al., 2014; Morgan, 2014). Most mortality usually occurs hours or a few days post-stress (Lockwood et al., 1983), but there may also be a delayed mortality which is not possible to capture in short-term experiments lasting days or weeks (Chopin & Arimoto, 1995; Misund & Beltestad, 2000; Marçalo et al., 2013).

Norway has introduced regulations banning release of fish in the later stages of purse seine hauling (§48a, Regulations Relating to Sea-Water Fisheries). If a catch is to be slipped, it must be released before 7/8 of the total length of the net is hauled (known as the 'point of retrieval') to minimize unaccounted mortality. For mackerel fisheries in the EU, this point of retrieval is set to 80% (i.e. 80% of the net has been hauled), after which it is prohibited to release the catch (EU Commission Discard Plans for the North Sea and North Western Waters, 2014). Purse seine gear is therefore fitted with a visible white buoy to set this limit. Despite these management efforts to mitigate the stressors within the purse seine capture process, the schooling fish are still unavoidably exposed to these stressors *before* their release, and could impact on their behaviour and physiology, and therefore survival – particularly in smaller and more vulnerable individuals (Boutilier et al., 1984; Chopin & Arimoto, 1995; Marçalo et al., 2013).

1.3. MACKEREL BIOLOGY

Figure 2. Atlantic mackerel *Scomber scombrus* (Linnaeus 1758). Source: norpel.com.

Atlantic mackerel, *Scomber scombrus* L. (Figure 2), is a highly migratory schooling fish species found most commonly in the North Atlantic and Mediterranean Sea. The North-east Atlantic population is separated into two stocks: the eastern North Sea stock, and the western British Isles stock (Figure 3). They are abundant in cold and temperate shelf areas, overwintering in deeper waters but moving closer to shore as temperatures rise in the spring (Collette & Nauen, 1983). Although mackerel have a depth range of up to 1000m, they are usually found schooling close to the surface with better light conditions and prey availability (Collette & Nauen, 1983; Misund et al., 1996). Mackerel are piscivorous, feeding mainly on zooplankton and small fish.

Individuals can grow to a maximum of 60cm (Muus & Nielsen, 1999), although this is closer to 30cm in wild populations (Collette & Nauen, 1983). The maximum single weight of an individual mackerel was 3.4kg (Frimodt, 1995), and they can live as long as 17 years (Anderson & Paciorkowski, 1980). Many studies have been published following the feeding and spawning migration patterns of Atlantic mackerel (Bolster, 1974; Hamre, 1978; Holst & Iversen, 1992; Uriarte & Lucio, 2001; Iversen, 2002; Godø et al., 2004).

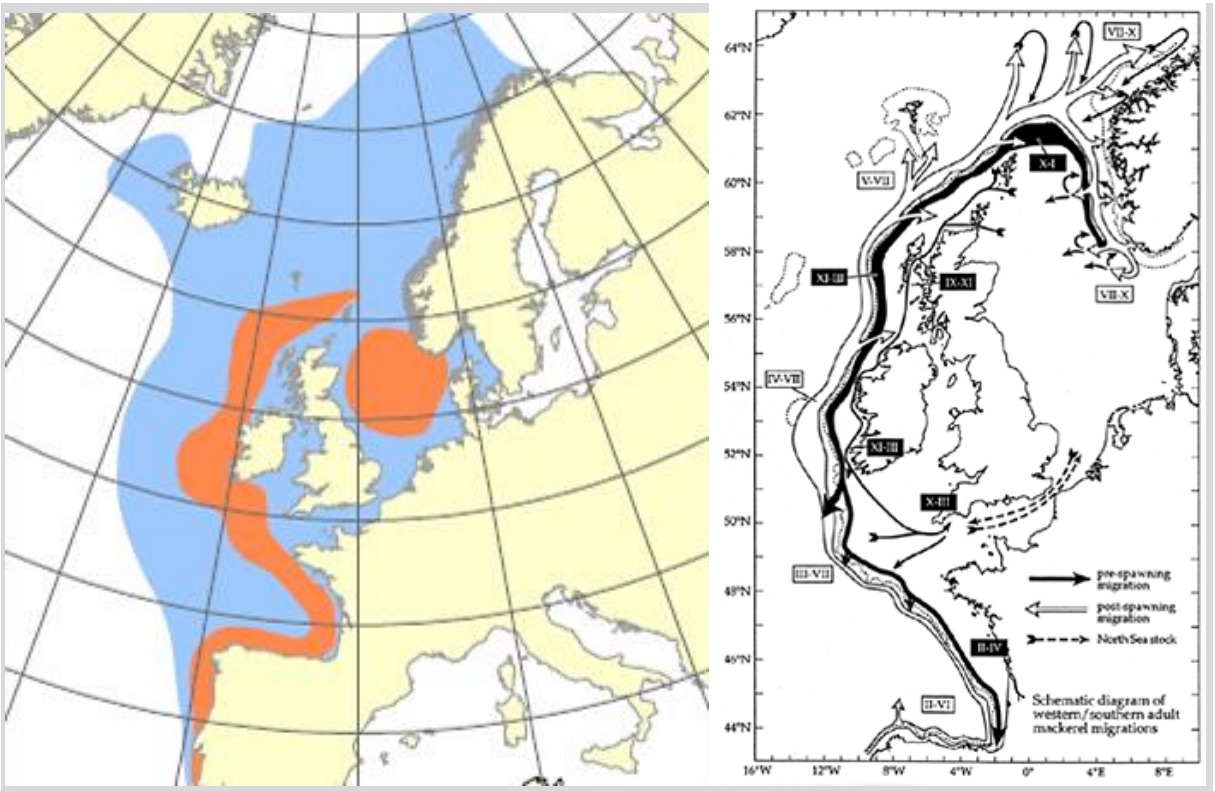

Figure 3a-b. a. Distribution of spawning grounds (orange) and feeding areas (blue) of Atlantic mackerel. Source: imr.no
b. Migration patterns of the North Sea Atlantic mackerel stock. Source: Reid et al., 1994.

Like other scombroid fishes such as tuna and bonitos, mackerel are also high performance carangiform swimmers (Tytell et al. 2010). They have a high proportion of red muscle (He, 1993; Altringham & Ellerby, 1999) and an optimal body shape for reducing drag (Wardle & He, 1988). Mackerel are capable of maintaining continuous high swimming speeds for long periods of time (Boutilier et al., 1984; He & Wardle, 1986; Godø et al. 2004; Nauen & Lauder, 2002), ranging from 1 to 3.5 body lengths per second without experiencing any exhaustion (He, 1993). Maximum speeds of mackerel of 18 body lengths per second have been recorded, although these speeds are highly unsustainable and result in rapid energy depletion (He, 1993). At low swimming speeds of around 20-60cm/s, mackerel utilize buccal ventilation, and switch to ram ventilation when swimming at faster speeds (Holeton et al., 1982; Boutilier et al., 1984).

Mackerel are unusual compared to other teleost fish in that they do not possess a swimbladder. As a result, mackerel are negatively buoyant, and must swim constantly to generate enough lift to avoid sinking (He, 1993; He & Wardle, 1986), although they are aided somewhat by tilt from their tail acting like a hydrofoil and providing extra vertical thrust (Wardle & He, 1988). This has made acoustic surveys of mackerel schools problematic in the past, as the swimbladder is the organ where the source of most backscatter – approximately 90% - usually comes from (Foote, 1980; MacLennan & Simmonds, 1991; Reeder et al., 2004). Newer acoustic methods instead use higher frequencies to survey mackerel in order to provide the best frequency response from mackerel flesh (Gorska et al., 2005; Korneliussen & Ona, 2002).

1.4. SCHOOLING BEHAVIOUR

Behaviour represents a reaction to the environment as fish perceive it (Whitmarsh & Young, 1985; Martins et al., 2012). As a quick and external response, behaviour has provided a key element of fish welfare for investigating stress in individual fish and in schools (Dawkins, 2004). Behavioural metrics of stress are easier to identify, less intrusive to the fish and easier to measure *in situ* than physiological methods, therefore providing a greater likelihood of survival if responses are identified earlier on (Dawkins, 2004; Korte et al., 2007; Schreck, 2010). Behaviour can be observed in two contexts – as school behaviour, and as behaviour of individuals.

A school is described as a synchronized, polarized aggregation of fish (Pitcher, 1983; Pitcher & Parrish, 1993; Lopez et al., 2012). Approximately 25% of species show schooling behaviour at some point throughout their life (Shaw, 1978). Schooling behaviour is common in all cluepid (herring) and scombroid (mackerel) species, typically choosing neighbours of similar size (Pitcher et al., 1985; Misund, 1988). Animals living in groups make movement decisions depending on social interaction between group members (Pérez-Escudero & de Polavieja, 2011). In a similar way, schooling enables individuals to maximize the flow of information about swimming behaviour between neighbours from either visual or lateral line cues, usually to rapidly transfer threat information to other fish, such as an oncoming predator (Partridge et al., 1980; Lopez et al., 2012; Rieucau et al., 2014a; Brierley & Cox, 2015). Density and internal organization of a fish school affects the extent to which information can transfer through the school, consequently affecting the strength of these collective behavioural responses (Rieucau et al., 2014b).

Schooling behaviour has many benefits for fish. Schools provide protection from predation (Brierley & Cox, 2010; Marçalo et al., 2013), and hydrodynamic efficiency (Weihs, 1973; Herskin & Steffenson, 1998; Killen et al., 2012; Hemelrijk et al., 2015; Marras et al., 2015). The 'selfish shoal' hypothesis suggests that the bigger the group, the less chance of predation on an individual, making it more advantageous to be in a larger group than a smaller group (Hamilton, 1971; Parrish, 1989; Brierley & Cox, 2010). However, schooling also makes more attractive targets to fishers and predators, as schools are easier to find and offer much higher yield than dispersed individuals (Rieucau et al., 2014a; Brierley & Cox, 2015). Despite avoidance behaviour from vessels, fishing has adapted with gear designed to manipulate fish behaviour in ways to facilitate capture. Purse seine fisheries utilize avoidance behaviour to herd fish for capture (Davis 2002; Handegard et al., 2014; Rieucau et al., 2015).

Lopez (et al., 2012) suggested schooling is governed by three basic behavioural rules:

Cohesion	The attraction rule	This enables fish to group with conspecifics in order to produce aggregation. Vision drives this rule. (Pitcher & Parrish, 1993).
Directional orientation	The alignment rule	Fish match the behaviour of their neighbours in allelomimetric behaviour.
Collision avoidance	The repulsion rule	Fish maintain a certain distance from their nearest neighbour. Lateral line drives this rule. (Pitcher & Parrish, 1993). This takes the highest priority.

These rules affect the school internal structure, including horizontal and vertical distribution, and density. Polarity is affected by the rule of alignment, while inter-individual spacing are characteristics driven by two opposite forces – attraction, driven by vision; and repulsion, driven by the lateral line system (Pitcher & Parrish, 1993; Gueron et al., 1996; Parrish et al., 2002; Tien et al., 2004). School structure is considered to be disrupted when fish do not show uniform orientations and are swimming in different directions (Domenici et al., 2000).

Schooling behaviour, including school size and distribution, is driven by both biological and environmental conditions, such as temperature, oxygen, light and food availability (Whitmarsh & Young, 1985; Glass et al., 1986; Scalabrin & Masse, 1993; Fréon & Misund, 1999; Weetman et al., 1999; Domenici et al., 2002; Mori & Boyd, 2004; Bertrand et al., 2006; Domenici et al., 2007; Brierley & Cox, 2010; Marçalo et al., 2013).

The 'compressing-stretching-tearing' hypothesis (Fréon et al. 1992) suggests that inter-fish distances and polarization level depends on state of environment. In a low stress environment, fish show individualist and exploratory behaviour, increased inter-fish distances and lower

polarization, as seen in Figure 4a (Azzali et al., 1985; Fréon et al., 1996; Fréon & Misund, 1999; Bertrand et al., 2006). This is typically seen in mackerel during the night, when school structure loosens as individuals tend towards individual food-search behaviour with higher prey availability (Bertrand et al., 2004, 2006; Brehmer et al., 2007). This behaviour has been seen in schooling minnows (Robinson & Pitcher, 1989a), and herring (Morgan, 1988; Robinson & Pitcher, 1989b). Alternatively, high stress environments - such as proximity to predators or during the capture process - results in denser schools of fish swimming closer together and greater polarization.

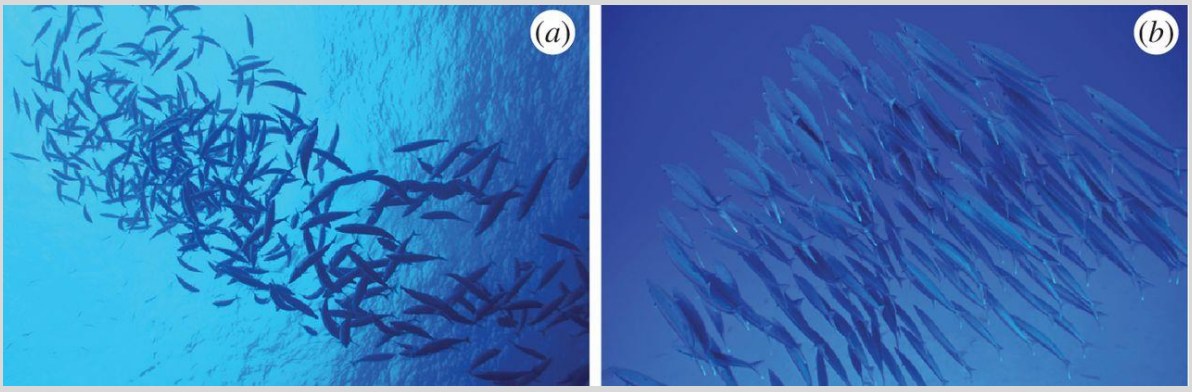

Figure 4. School behaviour that is poorly (a) and highly (b) polarized. Source: Lopez et al. 2012

School behaviour is the collective behaviour of all individuals within the school. As such, a single individual fish can alter the behaviour of the whole group (Romey, 1996; Domenici et al., 2002; Martins et al., 2012). This makes it important to look at the specific behavioural mechanisms taking place at the individual level. Individual swimming performance has previously been used in behavioural studies as a proxy for effort (Herskin & Steffensen, 1998; Huntingford et al., 2006; Morgan, 2014). Metrics including tail beat frequency and tail beat amplitude have been used to study individual fish behaviour and swimming speeds in schooling species (Bainbridge, 1958; Beamish, 1978; Wardle & Videler, 1980; Videler & Hess, 1984; Morgan, 2014; van Weerden et al., 2014). Typically, sustained steady swimming is characterized by low-frequency tail beats and a slow velocity, while swimming when exposed to stressors can result in fish almost reaching their maximum velocity (Lembo et al., 2007). Oxygen availability can affect swimming activity (Randall, 1970; Bryan et al., 1990; Herskin & Steffensen, 1998; Domenici et al., 2000). In most cases, fish swimming activity increases for a short period of time, as seen in cod, *Gadus morhua* (Schurmann & Steffensen, 1994) and herring, *Clupea harengus* (Herbert & Steffensen, 2006; Domenici et al., 2000, 2013) following exposure to hypoxic conditions. This could show a trade-off between fish respiratory distress at lower oxygen levels, and the need to find more favourable conditions.

1.5. STRESS RESPONSES

When fish are exposed to stressors, such as crowding or hypoxia, observable changes in the school and individual swimming behaviour can be used as indicators of stress level or welfare (Barton, 2002; Huntingford et al., 2006).

Stress is a threat to or disturbance of allostasis (Iwama et al., 2011). A stress response is the response to a stressful environment with the purpose of restoring allostasis and ensuring the best chance of survival in a threatening situation (Barton & Iwama, 1991; Johnson et al., 1992 Pottinger, 2008). This evolved as an adaptive response to short-term or acute stressors – however, if exposure to stress is chronic or continuous, stress responses can become maladaptive and potentially harmful (Barton, 2002; Temming et al., 2002; Korte et al., 2005; Braithwaite & Ebbeson, 2014).

Stress responses can be categorised into primary, secondary and tertiary responses (Barton, 2002). Primary stress responses are neuro-endocrinological responses (Selye, 1973) involving neurologically stimulated releases of catecolamines and plasma cortisol levels (Wendelaar-Bonga, 1997; Barton, 2002; Duncan, 2005). The secondary stress response is primarily physiological – for example, adrenaline induces increased circulation to the gills and swimming musculature, while cortisol initiates the rapid breakdown of glycogen into glucose within the fish (Massabuau 2001, 2003; Barton, 2002). The purpose of this secondary response is to maintain the stress response, which is energetically costly (Wendelaar-Bonga, 1997), and to remove lactates from tissues and avoid any oxidative stress (Martins, 2012).

Tertiary stress responses are a whole-animal change in performance, including behaviour (Barton, 2002). Behaviour is a sensitive indicator to physiological and biochemical changes that occur in response to stress (Pottinger, 2008; Iwama et al., 2011), and are fast, easily observed responses, making them good indicators of welfare (Huntingford et al., 2006; Martins et al., 2012).

Change in swimming activity has been shown to be a general behavioural indicator of stress (He, 1993; Huntingford et al., 2006; Martins et al., 2012; Morgan, 2014). However, the exact responses can differ between species and the type and intensity of stressors (Domenici et al., 2000). While decreases of activity have been seen in sardine exposed to crowding (Marçalo et al., 2013) and cod exposed to hypoxia (Schurmann and Steffensen, 1994), herring showed increases in activity during crowding, and was found to be highly correlated to fish mortality (Morgan, 2014). This project will use tail beat amplitude and tail beat frequency as a proxy of fish swimming activity.

Another behavioural indicator of stress is a change in school structure (Domenici et al., 2007). Changes in density could show differences in inter-fish distance within the school, and could affect the schooling rules of repulsion (Lopez et al., 2012). In normal mackerel behaviour, mackerel maintain a set distance from one another known as the repulsion zone. High crowding densities in a purse seine force fish closer together, and create a stressor to fish behaviour by reducing the zone of repulsion from neighbours. Vertical distribution of a school can also change under stressful conditions, with most schools showing escape behaviour towards the bottom of tanks or cages when exposed to negative stimuli (Føre et al. 2009). This project will use vertical distribution and school density as a proxy of school structure.

1.6. AIMS AND OBJECTIVES

The main aim of this project is to determine potential behavioural changes (i.e. stress responses) of schooling mackerel with crowding and low oxygen (hypoxia) conditions in purse seine simulations. There are several research questions:

- Does individual mackerel swimming activity (tail beat frequency and amplitude) change with crowding and hypoxia?
- Does mackerel school structure and distribution (fish density and vertical position) change with crowding and hypoxia?
- Do stress responses in mackerel change over experimental time? Is there evidence of adaption to stressors during treatment, and/or recovery post-treatment?
- Can behaviour be used as an indicator for stress in mackerel, and is there a dominant behavioural metric for indicating stress to be used for welfare?

2. MATERIALS AND METHODS

2.1. BACKGROUND

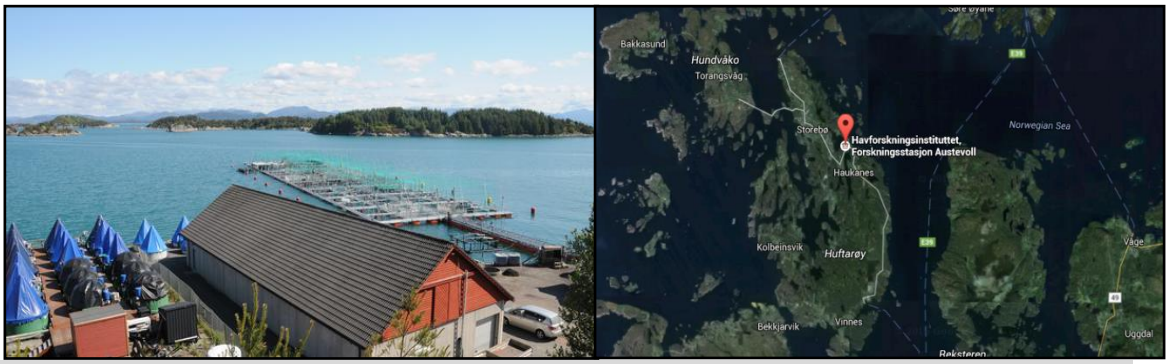

Figure 5. The fisheries research facility at Austevoll. Source: imr.no

The main crowding and hypoxia experiments were conducted on September 7th-17th 2015 at Austevoll Research Station, Norway (Figure 5).

Mattilsynet – the Norwegian Food Safety Authority (www.mattilsynet.no/), responsible for ensuring welfare of animals used in scientific research in Norway – specified that no stress-induced mortality of mackerel was allowed in the main experiments (§13 Animal Welfare Act, 2009). As a result, pilot experiments were carried out using the aquaria facilities at Austevoll from June 22nd to July 3rd 2015. These pilot studies observed mackerel schools under a range of crowding and hypoxic conditions in a controlled aquarium environment, with the objective of establishing safe stressor thresholds in preparation for the main experiments.

Both the pilot and main experiments were carried out as part of the Norwegian Research Council funded project "Reducing slipping mortality in purse seines by understanding interactions and behaviour" (REDSLIP, NFR 243885).

During the autumn of 2014, mackerel were captured in a standard aquaculture net-pen (12x12x10 metres), and were held and fed daily with aquaculture pellets at the Austevoll Research Station. One week before the start of the experiment, subsets of mackerel were transferred from the keeping pen into four experimental pens with dimensions of 5 x 5 x 6.5 metres (Figure 6). One of the experimental net-pens was used as a trial run for equipment and practicing crowding and hypoxia methods, while the other three were used in the main experiments. Predator avoidance experiments, that were also part of the REDSLIP project, took place within each of the experimental net-pens concurrently with the crowding and hypoxia experiments.

Figure 6. An experimental net-pen at the Austevoll Research Station. The dimensions are approximately 5x5x6.5 metres, with a pyramid-shaped base and a collection bag at the bottom for any dead fish.

A maximum number of approximately 500 individual mackerel (visual estimate) were kept in each net-pen. The experimental sub-samples were not so large as to be oxygen-limited, but not so small as to inhibit collective information transfer between individuals in the school (Brierley & Cox, 2015). Physical contact and unnecessary stress was seen to have had a negative impact on the behaviour of the fish in the pilot experiments, so this was carefully avoided. The mackerel were rested for seven days following the transfer to allow them to acclimatize to the experimental net-pens. The mackerel were not fed for 24 hours before and after the treatments, as well as when behavioural observations were made during the monitoring period, in order to prevent any individual feeding behaviours that might mask the stress response.

Samples of 30 individual mackerel were taken from each net-pen for length and weight measurements after the experiments were completed (Figure 7). These values were later used in the acoustic data analysis.

Figure 7. Individual mackerel were measured for length and weight. Photo courtesy of Eugene Kitsios.

2.2. TREATMENT GROUPS

Three experimental net-pens were used to simulate crowding and slipping events, each with different treatments (Table 1). An initial pre-treatment monitoring phase was used as a control in each net-pen, followed by three phases of experimental treatment – crowding & hypoxia, crowding, or a control treatment (where no stressors were applied to the net-pen).

Table 1. Experimental treatments and times for phases in each net-pen. P = pre-treatment monitoring; T1-3 = experimental treatment (numbers specify duration), M1-4 = post-treatment monitoring (numbers specify duration).

Phase	Day	Description	Hours after treatment		
			Net-pen 1 *Crowding & hypoxia*	Net-pen 2 *Crowding*	Net-pen 3 *Control*
P	0	*Pre-treatment monitoring*	-1	-2.5	-1
T1	0	*Start of treatment*	0-0.25	0-0.5	0-0.75
T2	0	*Treatment (ongoing duration)*	0.5-2	1-1.5	2-2.25
T3	0	*Treatment (ongoing duration)*	*NA*	1.5-2	3-3.25
M1	1	*Post-treatment monitoring*	28-29	26-27	23-24
M2	2	*Post-treatment monitoring*	47-48	44-45	49-50
M3	3	*Post-treatment monitoring*	70-71	70-71	70-71
M4	5	*Post-treatment monitoring*	142-143	143-144	142-143

Mackerel in Net-pen 1 were subjected to crowding and hypoxia over a period of approximately 2 hours – the net-pen was crowded to approximately half of its original volume, and oxygen levels were allowed to deplete over time to a minimum concentration of 40%. Mackerel in Net-pen 2 were crowded to approximately half of the original net-pen volume, but oxygen levels were not reduced (99-100%). Mackerel in Net-pen 3 were left as a control group – the net-pen was kept at its original volume throughout the experiment, and oxygen levels were not reduced (99-100%).

Crowding was simulated by pulling up the base of the net-pen, reducing the volume and increasing mackerel density over the span of approximately 2 hours (representing a common duration of hauling the purse seine). Ultimately, the net-pen volume was reduced by more than half (to approximately 1 metre from the bottom selvedge), determined by observing the seams of the net.

Oxygen depletion treatments were performed by surrounding the entire net-pen inside a large white delicing bag (typically used in aquaculture) to isolate the school from the water body, and allow oxygen to be consumed over time. In the non-hypoxic crowding treatment – Net-pen 2 – the net-pen was also enclosed in a bag, but was left sufficiently open at the surface as not to limit oxygen supply. This ensured that the behaviour of the mackerel was not affected by altering light conditions, as this has been previously shown to have a significant effect on avoidance behaviour in many fishes (Vowles et al., 2014), or from approaching predators that might startle the mackerel in the net-pen and produce additional stress or escape behaviour.

2.3. INSTRUMENTATION

Video recordings were obtained from a vertically-orientated camera, positioned looking up into the centre of each net-pen, while a stereo-camera system was placed in the inner corner with a horizontal view across the cage. The vertical camera was attached to a CTDO system (for measuring conductivity, temperature, depth and a probe for dissolved oxygen). This was then lowered into the middle and just below the school to try to get as many fish in the field of view as possible (Figure 8). The vertical camera faced upwards to provide the best contrast between the fish and the background light. This video footage was used to measure tail beat frequency and amplitude. An EK60 echosounder was placed at the bottom of the net-pen facing upward towards the school, in order to measure vertical distribution, density and biomass of the school.

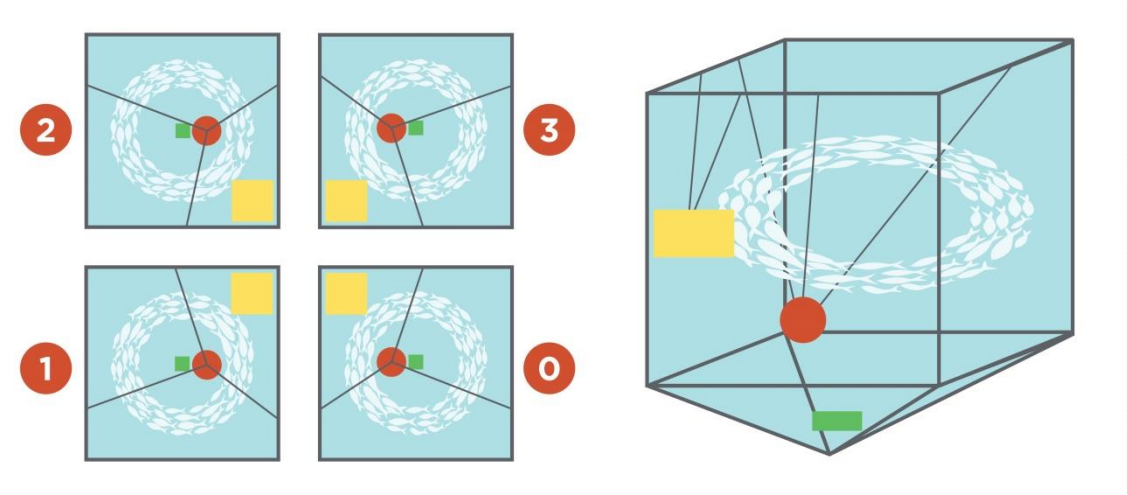

Figure 8a-b. a. Layout of the net-pens (**0** = Practice net-pen; **1** = Crowding & hypoxia; **2** = Crowding; **3** = Control;). **b.** Layout of equipment within the net-pen. Red = stereocamera system. Yellow = CTDO unit with vertical camera and oxygen probe. Green = EK60 echosounder.

2.3.1. VERTICAL CAMERA

Figure 9. GoPro Hero 3 camera with waterproof housing. Used in vertical video capture for tail beat frequency and amplitude analysis.

Video footage was obtained using a GoPro Hero 3 (Figure 9) – the fish-eye aspherical lens (aperture of f/2.8) and high resolution of video (capable of 12 Megapixel effective photo resolution) provided a wide angle with reduced distortion for a precise field of view (FOV value of 14mm). This made it highly suitable for observing a large number of fish in a contained area. The captured video dimensions were 1920 x 1080 (with a screen aspect ratio of 16:9) with a framerate of 30 fps. The camera was kept within a waterproof housing, capable of being submerged to a depth of 60 metres. Video from the GoPro was always time-synchronized with the master computer hub set to Greenwich Mean Time (GMT) by displaying the time and date on a watch or mobile phone before the camera was deployed.

2.3.2. ACOUSTIC DATA

Vertical distribution and density of the school was monitored using a SIMRAD EK60 scientific single beam echo sounder measuring acoustic backscatter energy from the mackerel school.

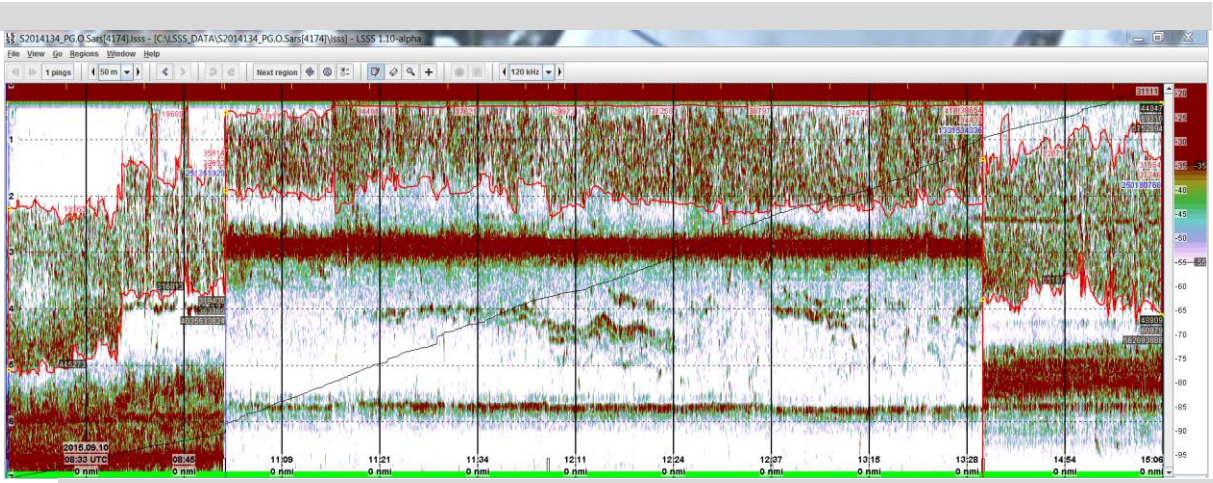

Figure 10. Example of an unprocessed echogram produced by LSSS (Large Scale Survey System) post-processing software.

The EK60 echosounder transmitted a pulse of sound directly upwards from the bottom of the net-pen towards the surface. The pulse of sound echoed off the mackerel or the surface, and returned downwards until the echo was detected by the echosounder. The time it took for the echo to return to the echosounder determined the range.

Each acoustic ping returned a specific value depending on the responding echo strength (Figure 10). The echo sounder operated at 120kHz, as this higher frequency provides the best relative

frequency response from mackerel flesh (Godø et al., 2004; Korneliussen, 2010). Acoustic assessment of mackerel also require higher frequencies to identify and correct for tilt angles compared to other teleost fishes, due to the lack of a swimbladder (He & Wardle, 1986; Gorska et al., 2007; Forland et al., 2014). The transducer was mounted at the bottom of the pen facing upward. The opening angle of the echo beam was 7°, determining the width of the sampled area. The pulse duration was 0.128 milliseconds, along beam resolution was 2.3 cm and the pulse rate was about 7 pings per second.

2.3.3. ENVIRONMENTAL DATA

A SAIV SD208 CTD (conductivity, temperature, depth) logging instrument with an additional Rinko III optode (for measuring dissolved oxygen) was the primary instrument for recording temperature, salinity and oxygen concentration in the water column during the treatments. This was placed underneath the school at the bottom of the net-pen so as not to disturb the 'natural' schooling behaviour. As a back-up for real-time monitoring, an oxygen probe was lowered into the centre of the net-pen, and was used to collect oxygen concentrations from the approximate centre of the school. These values were manually recorded and stored in MS Excel for use in the analysis.

2.4. DATA COLLECTION

The experiments were divided into three separate parts; pre-treatment (P), treatment (T) and monitoring (M). These parts were further subdivided into phases (Tables 1 & 2). The pre-treatment monitoring (Phase P) and treatment phases (T1, T2, T3) were all carried out over the space of one day (Day 0), with Phases T1-3 occurring during the specific treatment of each net-pen over increasing time (simulating the ongoing duration of a real crowding and slipping event). Monitoring phases M1-4 were set 1, 2, 3 and 5 days post-treatment for observations. Sequences were sampled randomly within the pre-determined time-frame of these phases. A full metadata table is included in Appendix 1.

Acoustic data were only collected for Phases P and T1-3, i.e. the pre-treatment monitoring and all three treatment observations on Day 0 (Table 2), due to instrument availability. Timings of phases in the acoustic data were coordinated to overlap with the video footage from both the vertical camera and stereocamera, and were kept to approximately 15 minutes each.

Table 2. Duration and days of each experimental phase used for the acoustic data collection.

Phase	Description	Hours after treatment		
		Net-pen 1 *Crowding & hypoxia*	Net-pen 2 *Crowding*	Net-pen 3 *Control*
P	*Pre-treatment monitoring*	-1.25 to -1	-2.25 to -2	-1 to -0.75
T1	*Treatment*	0.2-0.5	0-0.25	0.5-0.75
T2	*Treatment (with ongoing duration)*	1.75-2	1.25-1.5	2-2.2
T3	*Treatment (with ongoing duration)*	2.75-3	2-2.25	3-3.25

2.5. DATA MANAGEMENT AND ANALYSIS

2.5.1. SAMPLING OF VIDEO MATERIAL

Short sequences of video were extracted from over 16 hours of raw footage from the treatment and monitoring phases. A sequence duration of five seconds (or 150 frames) was taken from each phase of each net-pen. A total of 360 fish were sampled in the vertical camera samples, with five individual fish sampled per sequence.

Three five-second-long video clips were randomly selected from each phase and treatment using the Excel function RANDBETWEEN. Each video file was processed using Sony Vegas Pro video-editing software, and saved within non-descript folder directories to avoid observer bias. Scripting in this software allowed a timestamp to be created on each new trimmed file (Figure 11). Once each clip had been timestamped, it was converted into individual frames using video-to-JPG conversion software (Figure 12).

On the first still of every frame sequence, a grid was applied as an overlay, and five fish were selected via randomly generated coordinates. If no fish were present at the selected coordinates, another set of random coordinates were generated until a fish had been found (Figure 13). Using ImageJ 1.49, individual fish were followed through the video stills in sequence, and the same fish were used to measure tail beat frequency (by counting) and tail beat amplitude.

Figure 11. A timestamp was applied to each five-second clip using scripting options in Sony Vegas Pro.

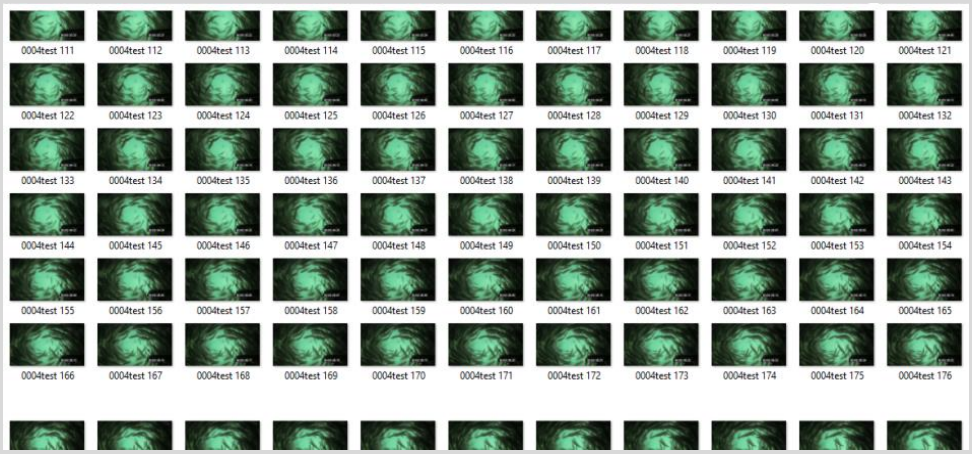

Figure 12. Each five-second clip was divided into individual frame sequences using video-to-JPG conversion freeware.

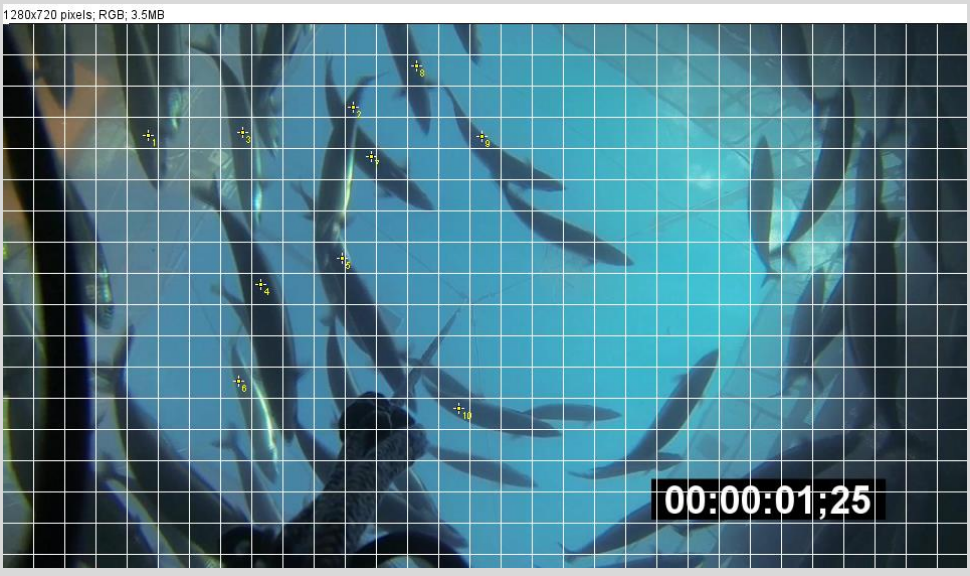

Figure 13. A grid overlay was applied in ImageJ. Random coordinates were used to assign individual fish in the first still image, and these were followed to count tail beat frequency and measure tail beat amplitude.

2.5.1.1. TAIL BEAT FREQUENCY

Tail beat frequency (TBF) refers to the number of times an individual fish has completed a tail beat (i.e. tail has reached the furthest distance from parallel to the line of the body) per second. Tail beats were manually counted from each randomly selected fish. If a fish was visible for over one second of video, then the number of tail beats was averaged over one second. If a fish was visible for less than one second of video, the number of tail beats was rounded up to one second.

An example of one complete tail beat is shown in Figure 14.

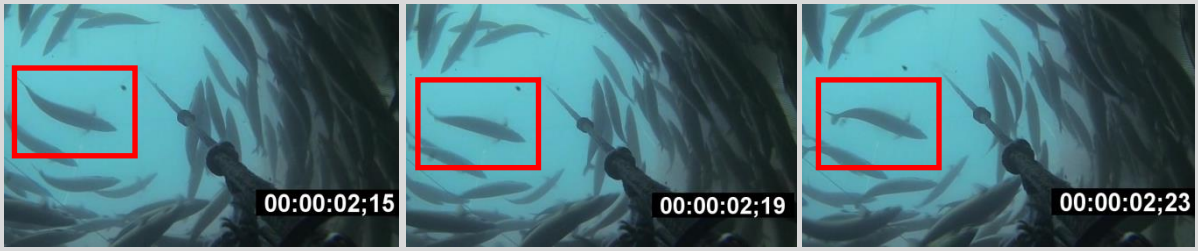

Figure 14. One complete tailbeat is shown by the mackerel individual in the red box. This was counted to give an average tail beat frequency per second, for each net-pen and for each phase.

2.5.1.2. TAIL BEAT AMPLITUDE

Tail beat amplitude is a measure of the lateral movement of the end of the tail with respect to the central axis of the direction of the movement of the fish. The tip of the tail describes an approximately sinusoidal path through the water (Figure 15). We call the amplitude of this sinus the tail beat amplitude (Videler & Wardle, 1991).

Figure 15. Progression of a tail beat and its change in amplitude. The shape of the tail beat is sinusoidal (represented by the dotted line). The largest amplitude is at the peak of this sinus, when there is maximal displacement of the tail tip from the back of the head. The mean of these maximal tail beat amplitudes was measured in each randomly sampled fish. Adapted from Akanyeti & Liao, 2013.

The maximum lateral displacement of each point of the body usually increases from just behind the head to the tail tip. The rate of increase differs among species (Videler, 1981). The total lateral excursion of the tail tip is usually the largest and hence it has the largest amplitude. The relative amplitude of the tail (amplitude over body length) is usually found to be constant over a wide range of swimming speeds, its value commonly being around 0.1 L. In other words, it is

the distance that the tail travels from the central line of the mackerel body at the point of a complete tail beat (Figure 16). This was measured using the ImageJ Line function to measure distance of tail from the body in pixels (Figure 17). The ratios of tail beat length to body length were measured for each tail beat, and then an overall average for each fish was taken, and this mean value was used in the analysis.

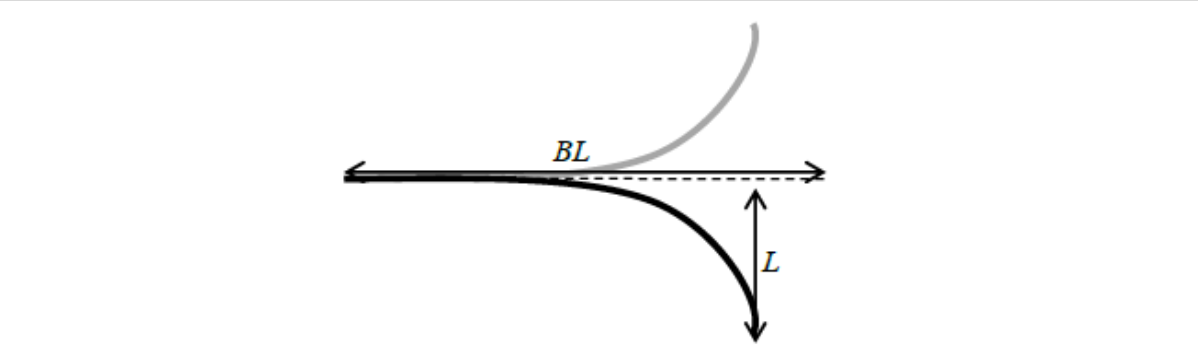

Figure 16. Calculating tail beat amplitude by measuring the length of half the tail beat (L) in pixels. The body length (BL) in pixels was measured and proportion was calculated using the formula L/BL (Videler & Wardle, 1991).

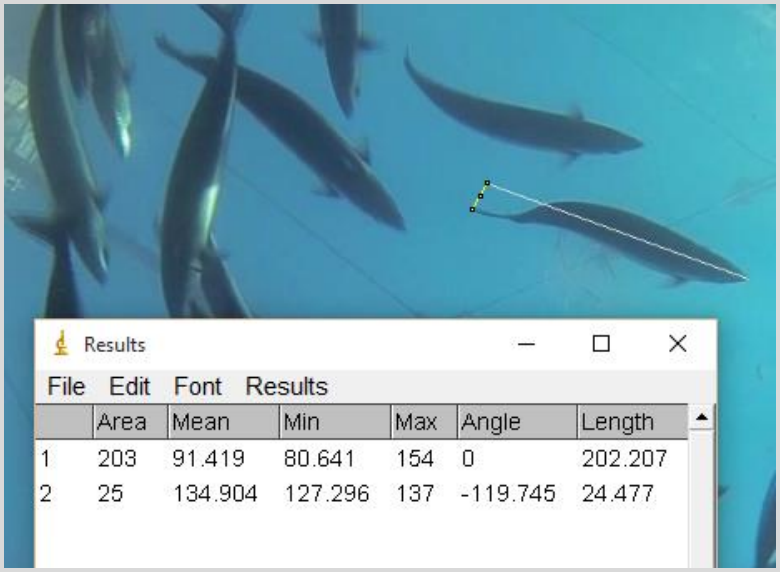

Figure 17. Calculating tail beat amplitude in ImageJ. The lines are assigned and then measured to give length values in pixels – the ratio of the two lengths gives the tail beat amplitude. The average TBA was calculated for each randomly selected individual.

2.5.2. ACOUSTIC DATA

2.5.2.1. PROCESSING IN LSSS

The acoustic data were processed using the Large Scale Survey System (LSSS) (Korneliussen et al., 2006). Echograms were produced for each net-pen in LSSS (Figure 18a-c), selecting the timeframe for the specific phases as listed in Table 2. This allowed for post-processing analysis in terms of vertical distribution, approximate biomass, and densities between phases and with each treatment.

Volume backscattering coefficients (S_v m^{-1} in decibels dB) were extracted from LSSS. S_v (or its linear form, s_v) is the amount of backscatter energy returned from an acoustic target. The stronger the s_v, the more backscatter is received from a volume, often indicating increased volumetric fish density. No differentiation was made between background noise and fish backscatter, but noise levels were assumed constant, and the stronger echoes were assumed to reflect mackerel.

All data outside the nearfield and up to the surface was extracted from the echograms. The surface was identified as the very strong backscatter energy (solid red colour) in the echogram (Figure 18). To reduce any noise in the data that might bias the analysis, data within the near field were excluded (Figure 18). The boundary between the near and far field (R_b) was calculated as 63cm using Equation 1, adapted from Simmonds and MacLennan, 2006.

$$R_b = a^2 / \lambda$$

Equation 1. Equation used to calculate the boundary between the near and far field.

λ = wavelength (1.3cm in seawater at 120kHz)
$a = 7\lambda$ = the linear distance across the transducer face

Acoustic area and volume backscattering coefficients (S_a m^2 m^{-2} and S_v m^{-1}) (MacLennan et al., 2002) were extracted by ping and by along beam samples and further analysed in R. The LSSS output file for the vertical distribution and density estimates included information on the ping number, frequency (120kHz), date and time, the start and stop distances for the range, the sample count (147), and the volume backscatter S_v values for each of the 147 samples along the range. The LSSS output file for the biomass estimates included information on frequency (120kHz), date and time, the depth and the area backscatter S_a values for each ping.

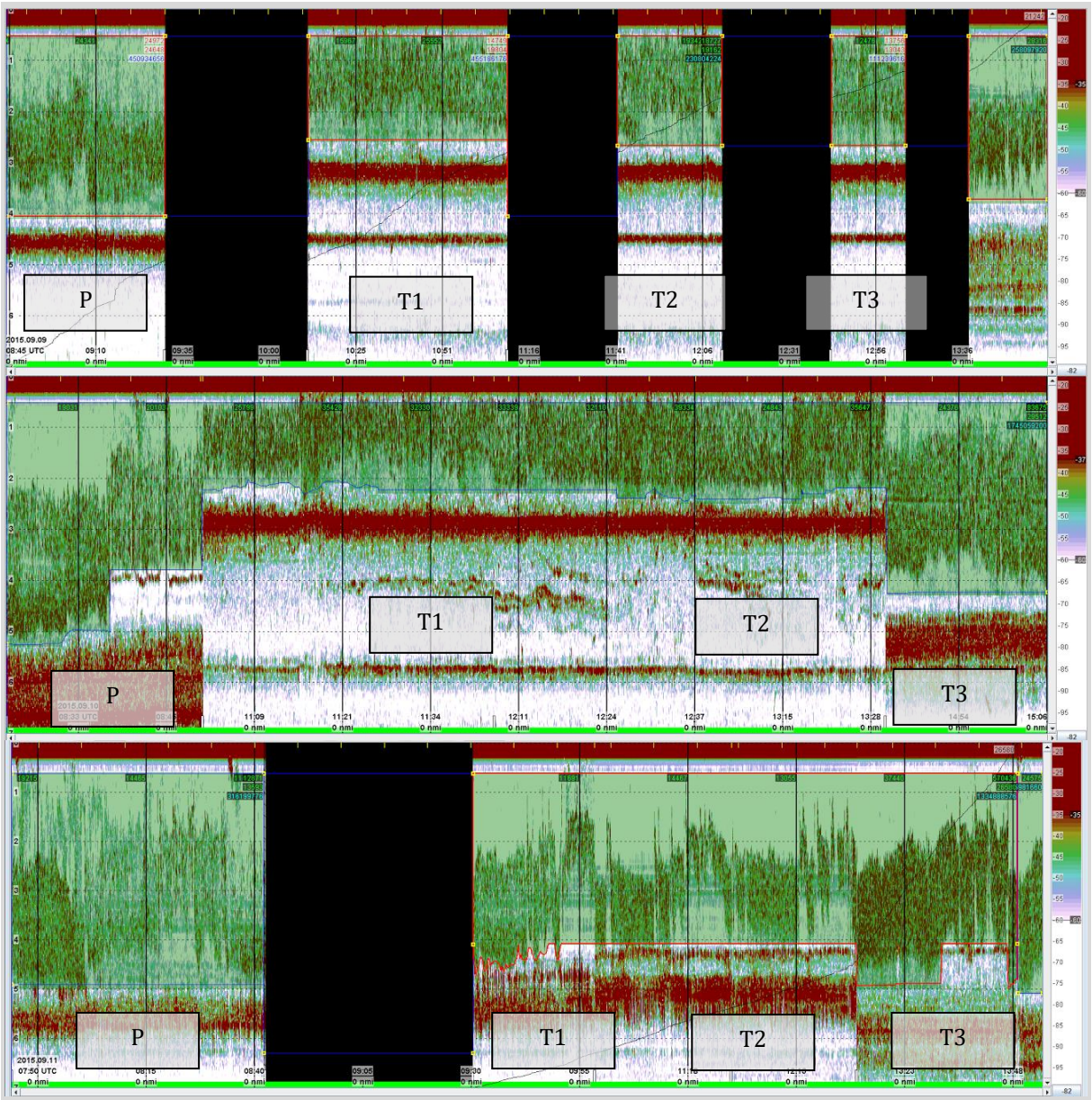

Figure 18a-c. Echograms from each net-pen. Phase P is the pre-treatment control and Phases T1-3 are during-treatment observations over time. Extracted data (assumed as the school) is outlined by the set solid red line. The strong backscatter signal is the echo from the surface. Dark red colours refer to backscatter that is stronger than -35dB, while lighter orange colours refer to backscatter strengths down to -60dB. The solid red colour in the echogram was used to identify the surface, and excluded from the exported data.

2.5.2.2. PROCESSING IN R

School vertical distribution, density and biomass were measured by phase and treatment. Due to the large data output from LSSS, data tables were rearranged to suit our analysis using the reshape2 package in R (Wickham, 2007).

Data were then separated into phases using the times outlined in Table 2. All backscatter data collected from the echosounder was averaged over the duration of the phase. The total depth of the net-pen within each particular phase was added, and the distance of the echosounder to the surface was determined by the range. Along-beam sample number was converted to distance from the echosounder using Equation 2. Distance from the echosounder was then converted into distance from the surface, a more ecologically meaningful measure, by subtracting the distance from echosounder from the total depth of the net-pen.

$$\frac{(Depth\ Stop - Depth\ Start)}{Total\ Sample\ Count} * Sample\ Number + Depth\ Start$$

Equation 2. Method for converting along-beam samples into distance from the echosounder.

Depth Stop = depth at which the beam ends (set in LSSS).
Depth Start = depth at which the beam starts (set in LSSS).
Total Sample Count = number of samples collected within the beam.
Sample Number = specific sample number along the beam.

S_v was then converted into linear (s_v) for analysis by using Equation 3 (MacLennan et al., 2002).

$$s_v = 10^{S_v/10}$$

Equation 3. Method for converting S_v (logarithmic value of backscatter energy) into s_v (linear value; MacLennan et al., 2002).

2.5.2.2.1. VERTICAL DISTRIBUTION

The vertical distribution (mean depth and spread) of the school between phases in relation to the surface (Equation 2) was analysed by observing the mean s_v m^{-1} value with depth (i.e. where the strongest backscatter energy came from).

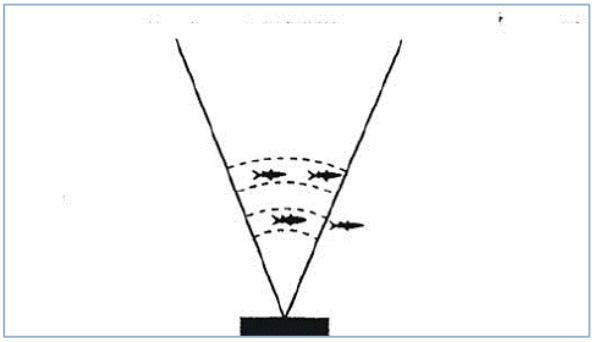

Figure 19. Transducer resolution and beam width. Fish further from the echosounder (black box) may return a weaker echo than fish closer to it, due to changes in beam width (dotted lines). Our data were thus weighted to compensate for this. Adapted from Brandt, 1996.

Data were averaged by depth layer over pings within each phase. This removed bias caused by the increasing beam width with distance from the echosounder – a wider beam-width has a higher chance of detecting fish (Figure 19). Each data point was weighted by the strength of the

echo using the Hmisc package in R (Harrell, 2016). Weighted means and weighted 5% and 95% quartiles of depth and s_v were calculated per phase (Equation 4a-b).

```
weighted mean <- netpen data [,wtd.mean(Depth, sv),by=Phase]
weighted quantiles <- wtd.quantile(data$Depth, data$sv, normwt=TRUE, probs=c(0.05, 0.95))
```

Equation 4a-b. R syntax using Hmisc package (Harrell, 2016) to apply weightings to s_v data by depth and phase. This provided outputs of weighted mean (4a) and weighted quantiles of 5-95% (4b).

2.5.2.2.2. DENSITY

Mean backscattering coefficient values per phase and depth (s_v m^{-1}) were then used to analyse school density per depth layer (number of individuals per volume).

The volume of the net-pen was first calculated for the rectangular section of the net-pen, plus the volume of the pyramidal section at the base (Figure 8b). Mean dissolved oxygen concentration during that time was also added, as well as proportion of the crowded net-pen to its uncrowded volume (%).

Target strength is a measure of the reflection coefficient of a sonar target, and it is important to know this in order to convert backscattering coefficients to fish density. This is usually quantified as a number of negative decibels. The constant value -82 refers to a logarithmic constant in decibels accounting for transmission loss (either from geometric spreading or absorption into the water column).

Firstly, target strength (*TS*) was calculated using Equation 5 (Simmonds and MacLennan, 2006). Average length of an individual mackerel for the equation was acquired from a length sample of 90 individuals, which was measured from each net-pen after the termination of the experiment.

$$TS = 20 * \log_{10}(\text{average length}) - 82$$

Equation 5. Equation used to calculate target strength (TS) of an individual mackerel for use in density estimates. *Average length* refers to average length of an individual mackerel in each net-pen. The constant -82 refers to a logarithmic constant (dB) accounting for transmission loss.

Target strength was converted to a linear value in order to get the backscattering cross-section (*bs*) using Equation 6.

$$bs = 10^{TS/10}$$

Equation 6. Equation used to linearize target strength (*TS*) to obtain backscatter coefficient (*bs*) values.

Density (n/m^3) was then calculated by dividing the backscatter energy (s_v) by the backscatter cross-section (*bs*), as shown in Equation 7.

$$density\ (n/m^3) = s_v / bs$$

Equation 7. Equation used to estimate density from each depth layer
s_v = backscatter energy (linear value).
bs = backscatter coefficient (calculated in Equation 4).

This method was applied to all net-pens, and density was plotted against net-pen. Data were grouped by phase in these plots.

2.5.2.2.3. BIOMASS

The sum of all the s_v values (s_a) gave estimates of biomass per net-pen (kg/m^2) – this was to ensure that there were similar biomasses between each net-pen, for a fair comparison of crowding density estimates. Biomass was estimated to ensure that there were similar biomasses of mackerel between each net-pen, thereby checking that similar behaviours were to be expected within each net-pen.

Biomass for each net-pen was estimated using the s_a ($m^2\ m^{-2}$) values extracted from LSSS (Equation 8).

$$bs = 4\pi * 10^{TS/10}$$

Equation 8. Equation used to calculate backscatter coefficient (*bs*) from target strength (*TS*) in biomass estimations.

Biomass was then estimated using the Equations 9a-d below (adapted from FAO, 2000).

$$density/nm^2 = S_a / bs$$

$$biomass/nm^2 = [density/nm^2] * mean\ individual\ weight\ (kg)$$

$$biomass/m^2 = [biomass/nm^2] / 1852^2$$

$$biomass/netpen = [biomass/m^2] * 5$$

Equation 9a-d. Steps for estimating biomass using area backscatter energy (S_a) values.

This process was repeated for all three net-pens, and plotted together.

2.5.3. STATISTICAL ANALYSIS

Microsoft Excel was used for data management, and all statistical analysis and plot creation used the RStudio statistical software (R v3.2.2; R Core Team, 2015).

Various models were fitted to the tail beat frequency data and explanatory factors, Phase & Treatment. The Gaussian (normal) distribution in a generalized linear model (GLM; Crawley, 2012) i.e. equivalent to a two-way ANOVA was found to provide the best fit, with only the four most extreme (two smallest and two largest) residuals deviating from the predicted error distribution (Appendix 2). GLMs (using Gamma distribution, with various link functions: inverse, log and identity) were also explored because of the non-normal nature of the raw data (i.e. skewed to the right with higher values). However, these were dropped in favour of the Gaussian GLM (ANOVA) because of the improved distribution of the model residuals (both in terms of normality and heteroscedasticity (i.e. non-uniform variance of a variable)). Plots of the GLM model fit, along with a plot of residual vs. fitted values, standard deviation of the residuals, and a quantile-quantile (QQ) plot have been all included in Appendix 2 (Zuur et al. 2009).

A two way ANOVA (analysis of variance) was used to compare levels of two explanatory factors (Phase and Treatment) for mean differences on a single continuous response variable (tail beat frequency, or tail beat amplitude).

Following the two-way ANOVA test, a post-hoc Tukey HSD (Honest Significant Difference) test (from the R *stats* package (R Core Team, 2016)) was used to compare each phase and treatment and any interactions between the two explanatory factors as a single-step multiple comparison procedure. This analysis also included a Bonferroni-type adjustment of the resultant p-values to reduce the risk of Type I inference errors from multiple comparisons.

Due to a lack of replicates, acoustic data could not be analysed statistically. As acoustic data were only collected once, and then data were averaged over both depth layer and phase, there are no other replicates, thus no more mean values or other variability in the data available for statistical comparison. As a result, the acoustic data will be presented only for observational, descriptive purposes.

3. RESULTS

3.1. VERTICAL CAMERA

3.1.1. TAIL BEAT FREQUENCY

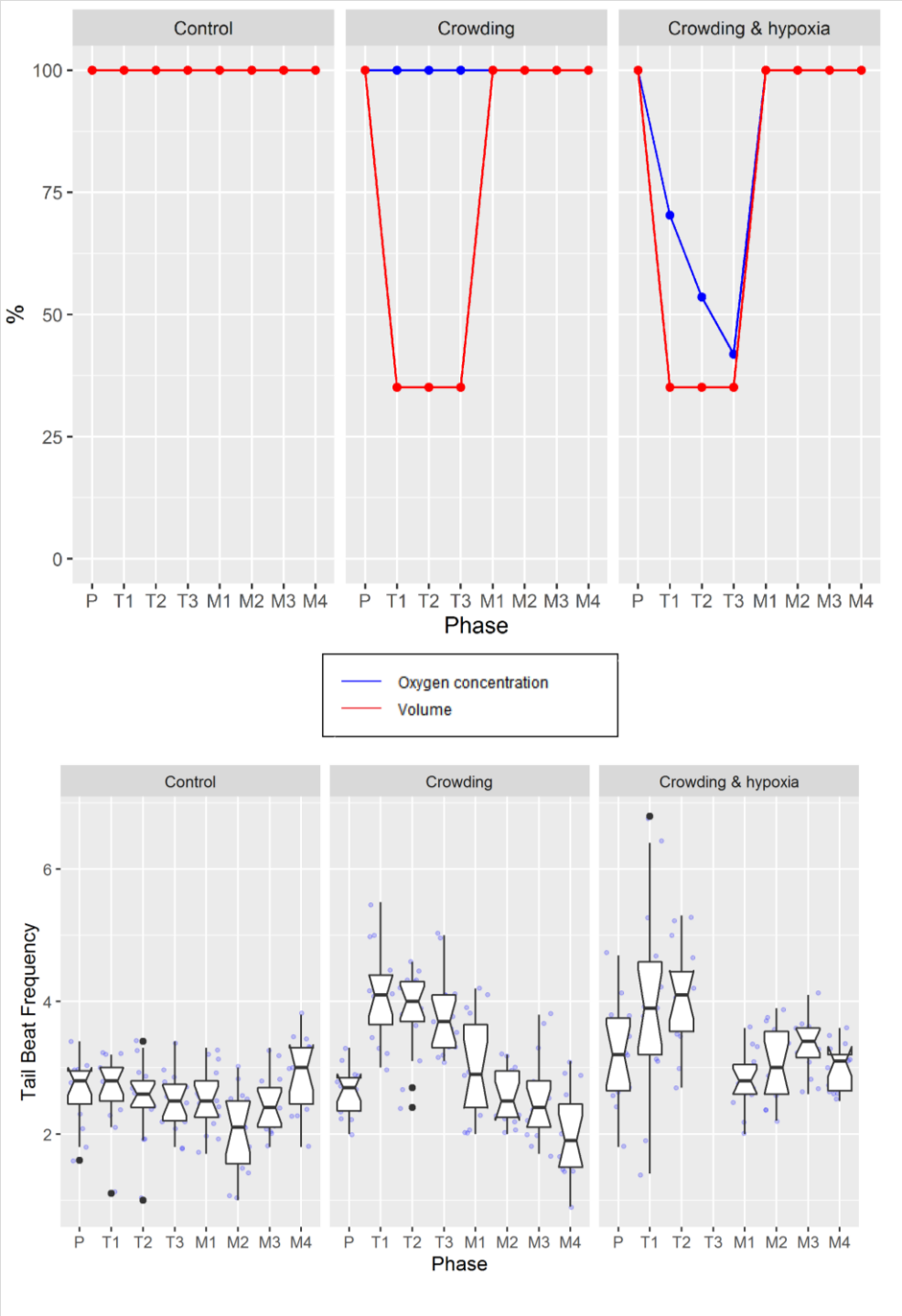

Figure 20. Tail beat frequency with treatment and phase. Individual data points (blue) show raw data (jittered for clarity, and not as an indication of time). Filled black circles show outliers. If notches do not overlap, there is a 95% likelihood that the groups differ. Box shows lower quartile (25%), median (middle line) and upper quartile (75%). Whiskers are 1.5x interquartile range. (Chambers et al., 1983).

Table 3. Analysis of variance (ANOVA) output table.
(Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1

Tail beat frequency	Degrees of freedom	F-value	Pr(>F)	Significance
Treatment	2	51.3802	$<2.2e^{-16}$	***
Phase	7	17.9991	$<2.2e^{-16}$	***
Treatment:Phase	13	7.3752	$1.088e^{-12}$	***
Residuals	322			

The oxygen and net-pen volume in the control treatment were unchanged throughout the experiment, with 100% dissolved oxygen concentration and the net-pen volume kept at 154m^2. In the crowding treatment, dissolved oxygen concentration remained at 100%, while the net-pen was decreased by over half for the treatment phases, from 154m^2 (P) to 54m^2 (T1-T3), before returning to 154m^2 for the monitoring phases (M1-M4). In the crowding and hypoxia treatment, the net-pen volume was reduced to 54m^2, while the oxygen concentration decreased over the treatment phases from 70.3% in T1, to 53.6% in T2, to 41.9% in T3.

Both treatment and phase had a significant effect on tail beat frequency. There was also a significant interaction between treatment and phase (Table 3). Tail beat frequency appeared to be higher during treatment phases compared with control and pre-treatment, before reducing again in the monitoring phases. The crowding treatment phases showed higher tail beat frequency than the crowded and hypoxia treatment phases. A table of Tukey HSD test output values is included in Appendix 3.

No significant differences were found between the pre-treatment phases P of all treatments (TukeyHSD, all p-values >0.05). Tail beat frequency did not change significantly from phase to phase in the control experiment (TukeyHSD, all p-values > 0.05).

In the crowding treatment, significantly higher tail beat frequencies were seen in all treatment phases compared to the pre-treatment phase (T1-T3; TukeyHSD, all p-values < 0.001). All tail beat frequencies in the monitoring phases were significantly lower than the first treatment phase T1 (M1-M4, all p-values < 0. 001) and the second treatment phase T2 (M1-M4, all p-values <0.05). Although the third treatment phase T3 did not decrease significantly in M1 (TukeyHSD, p=0.0555), the decreasing tail beat frequencies continued throughout the rest of the monitoring period (M2-M4, TukeyHSD, all p-values <0.0001). The tail beat frequencies during the monitoring period steadily decreased, with the only significant difference found between the start M1 and end M4 of monitoring (TukeyHSD, p=0.0064). No change in tail beat frequency was observed when comparing the pre-treatment phase to any of the monitoring phases (TukeyHSD, all p-values > 0.05), suggesting that TBF had returned to its original state following crowding.

In the crowding and hypoxia treatment, tail beat frequency in the pre-treatment phase was not found to be significantly different from any other phase, due to its high variance. Although high tail beat frequencies were seen in the treatment phases T1 and T2, there was no significant difference between the two, despite the duration and decreasing oxygen levels. Tail beat frequencies decreased significantly in the first two monitoring phases M1 and M2 compared to the first two treatment phases M1 and M2 (TukeyHSD, all p-values < 0.01), and between the second treatment phase T2 and last monitoring phase M4 (TukeyHSD, p=0.0017).

Higher tail beat frequencies were found in the treatment phases between the crowding (T1-T3) and the crowding and hypoxia treatment (T1-T2) when compared to the control experiment (TukeyHSD, all p-values <0.00001). No significant difference in tail beat frequency was found in the first phase of monitoring M1, but some increases were seen in the monitoring phases M2 and M3 in the crowding and hypoxia treatment compared to the control (TukeyHSD, all p-values <0.05). Significantly lower tail beat frequencies were found in the last monitoring phase M4 of the crowding treatment when compared to the control and crowding and hypoxia treatments (TukeyHSD, all p-values <0.05).

3.1.2. TAIL BEAT AMPLITUDE

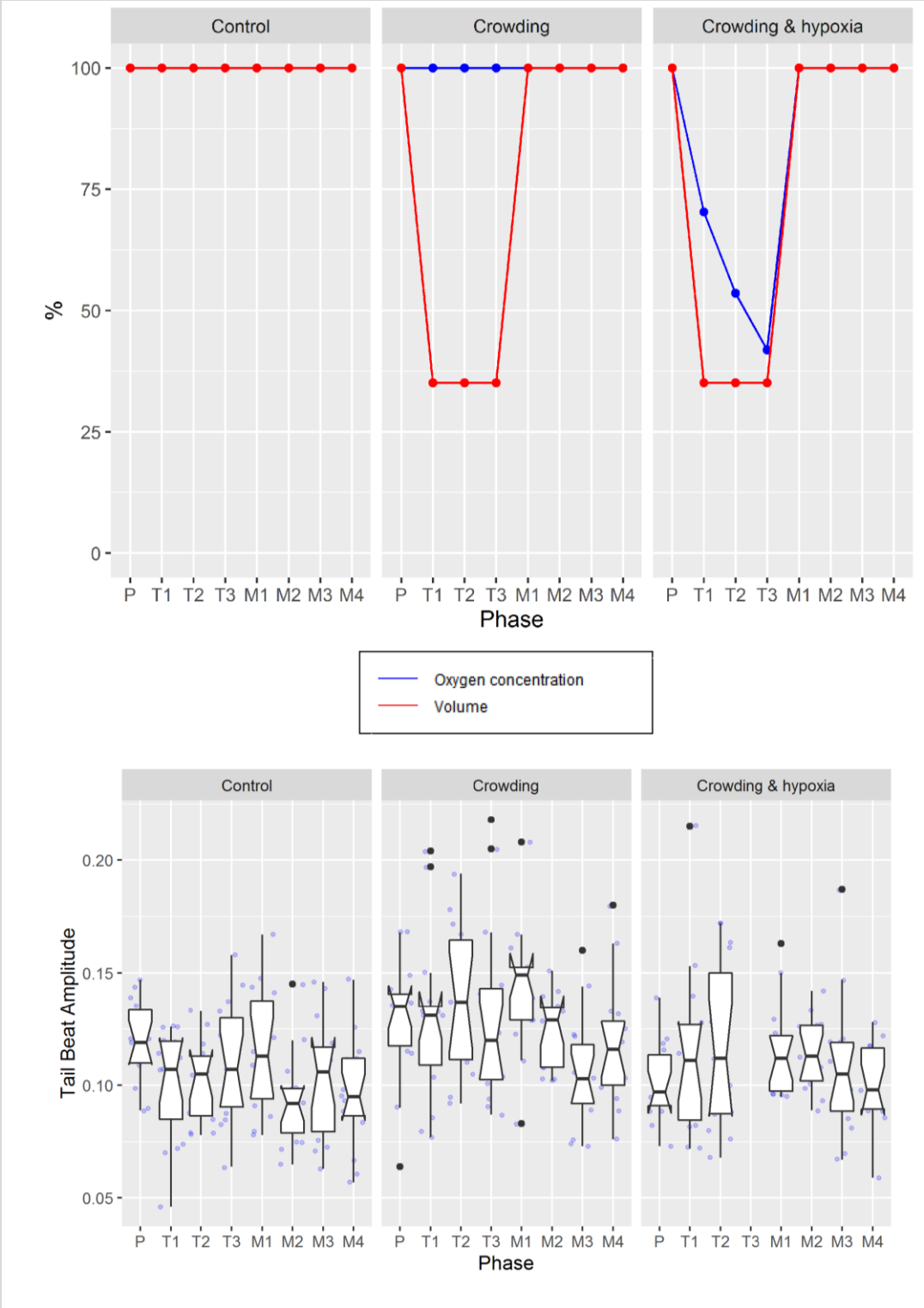

Figure 21. Tail beat amplitude with treatment and phase. Individual data points (blue) show raw data (jittered for clarity, and not as an indication of time). The box represents the interquartile range (IQR) accounting for 50% of the data. The solid black line in each box represents the median value. The upper whiskers account for the 75% quartile, and lower whiskers are the 25% quartile of the data. Filled black circles represent outliers. The 'notch' in each bar is the 95% confidence interval of the median (median +/- 1.57xIQR/$n^{0.5}$) (Chambers et al., 1983).

Table 4. Analysis of variance (ANOVA) output.
(Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1)

Tail beat amplitude	Degrees of freedom	F value	Pr(>F)	Significance
Treatment	2	22.0767	$1.035e^{-09}$	***
Phase	7	3.0317	0.004223	**
Treatment:Phase	13	1.3468	0.184324	
Residuals	322			

Both treatment and phase were found to have a significant effect on tail beat amplitude (Table 4). However, no significant interaction was found between treatment and phase. A table of Tukey HSD test output values is included in Appendix 4.

No significant change in tail beat amplitude was seen from phase to phase in the control experiment, or in the crowding and hypoxia treatment (ANOVA, all p-values > 0.05).

The only change in tail beat amplitude was a decrease during the crowding treatment, when comparing the first monitoring phase M1 with the penultimate monitoring phase M3 (TukeyHSD, p=0.0287). No other significant duration effects were seen in the crowding treatment (TukeyHSD, all p-values > 0.05).

A significant increase in tail beat amplitude was found when comparing the second treatment phase T2 between the crowding treatment and the control (TukeyHSD, p=0.0249). No other significant differences were seen between treatments.

3.2. ACOUSTICS

3.2.1. VERTICAL DISTRIBUTION

3.2.1.1. CONTROL

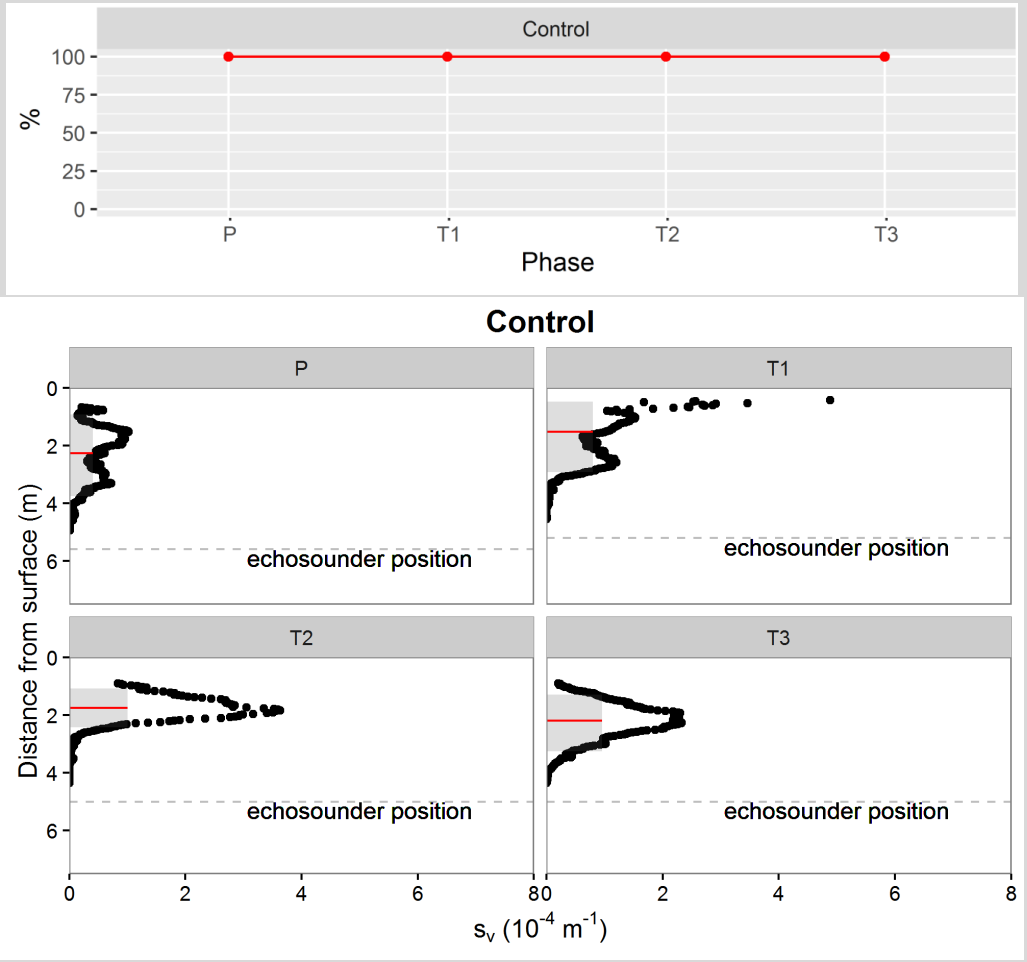

Figure 22. Backscattering coefficient (s_v m^{-1}) from the mackerel school averaged over time and plotted against distance from the surface in the control net-pen treatment. Each data point represents the average s_v value per depth layer. Echosounder position is shown by the dashed grey line. The solid red line indicates the mean depth of the mackerel school. The vertical width of the grey box indicates the 5-95% quantiles (i.e. the spread) of the school, while the horizontal width indicates the overall mean s_v value (i.e. backscatter coefficient). % Dissolved oxygen concentration (blue) and % volume of net-pen (red) with phase are plotted above.

The oxygen and net-pen volume in the control treatment were unchanged throughout the experiment, with 100% dissolved oxygen concentration and the net-pen volume kept at 154m^2. The weighted mean depth of the school in the control does not change; 2.3m in P, 1.5m in T1, 1.7m in T2 and 2.2m in T3. (Figure 22). However, the spread of the school decreases, from 2.9m in the pre-treatment P to 2.4m in phase T1, to 1.3m in phase T2. However, the spread increases again at the end of the treatment phase T3 to 2m. Mean volume backscattering coefficient showed an increase from pre-treatment values at the start of the treatment ($4 \cdot 10^{-5}$ m^{-2} in Phase P to $8 \cdot 10^{-5}$ m^{-2} in Phase T1), increasing to $1 \cdot 10^{-4}$ m^{-2} in Phase T2 before decreasing to $9.5 \cdot 10^{-5}$ m^{-2} in T3.

3.2.1.2. CROWDING

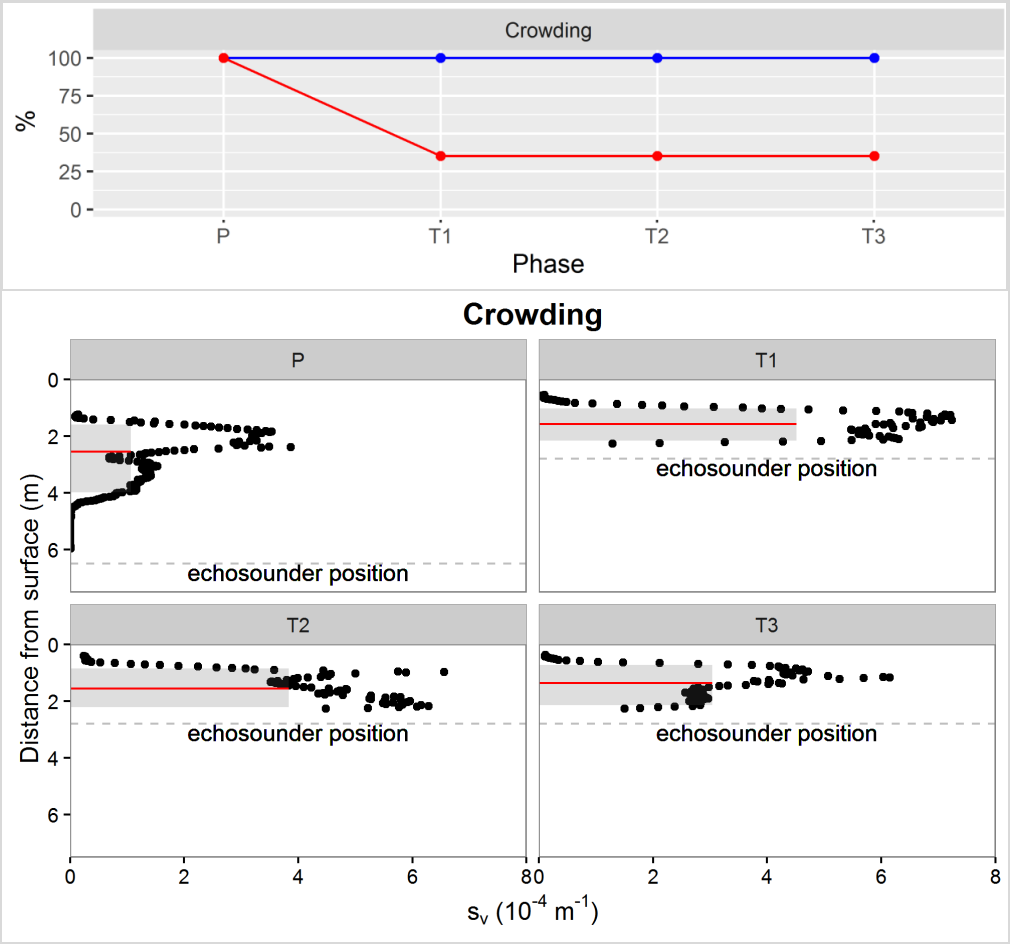

Figure 23. Backscattering coefficient (s_v m^{-1}) from the mackerel school averaged over time and plotted against distance from the surface in the crowding net-pen treatment. Each data point represents the average s_v value per depth layer. Echosounder position is shown by the dashed grey line. The solid red line indicates the mean depth of the mackerel school. The vertical width of the grey box indicates the 5-95% quantiles (i.e. the spread) of the school, while the horizontal width indicates the overall mean s_v value (i.e. backscatter coefficient). % Dissolved oxygen concentration (blue) and % volume of net-pen (red) with phase are plotted above.

In the crowding treatment, dissolved oxygen concentration remained at 100%, while the net-pen was decreased by over half for the treatment phases, from 154m² (P) to 54m² (T1-T3). Mean depth of the school during the crowding treatment changes from 2.6m in pre-treatment P to 1.6m during Phases T1 and T2, to 1.4m in T3 (Figure 23). The spread of the school (shown by the grey box of interquartile distance q_5-q_{95}) greatly reduces after the pre-treatment P (P IQR= 2.4m, T1 IQR= 1.2m, T2 IQR = 1.3m, T3 IQR = 1.4m), suggesting large changes in school density. This is supported by the mean volume backscattering coefficient – an increase from pre-treatment values at the start of the treatment ($1 \cdot 10^{-4}$ m^{-2} in Phase P to $4.5 \cdot 10^{-4}$ m^{-2} in Phase T1), before a steady decrease over the duration of the treatment ($2.5 \cdot 10^{-4}$ m^{-2} in Phase T2, and $1.75 \cdot 10^{-4}$ m^{-2} in Phase T3).

3.2.1.3. CROWDING AND HYPOXIA

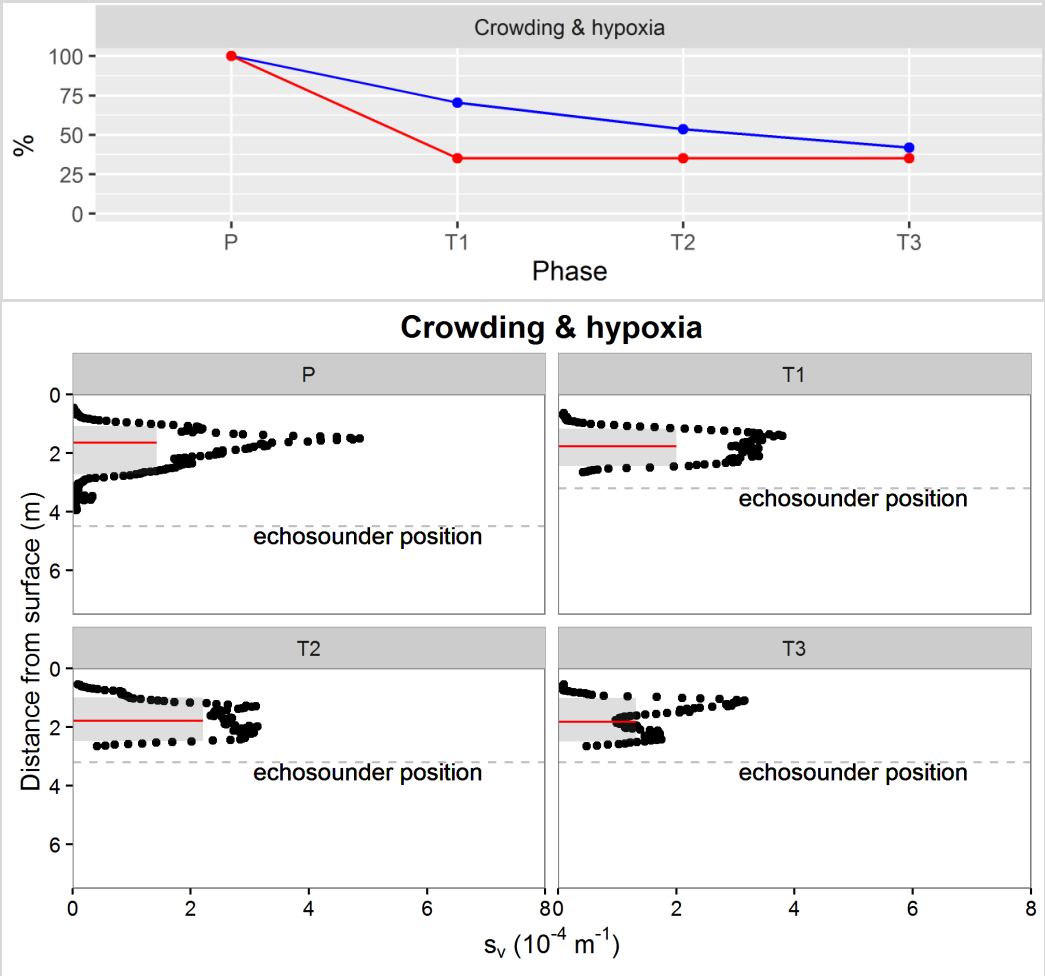

Figure 24. Backscattering coefficient (s_v m^{-1}) from the mackerel school averaged over time and plotted against distance from the surface in the crowding and hypoxia net-pen treatment. Each data point represents the average s_v value per depth layer. Echosounder position is shown by the dashed grey line. The solid red line indicates the mean depth of the mackerel school. The vertical width of the grey box indicates the 5-95% quantiles (i.e. the spread) of the school, while the horizontal width indicates the overall mean s_v value (i.e. backscatter coefficient). % Dissolved oxygen concentration (blue) and % volume of net-pen (red) with phase are plotted above.

In the crowding and hypoxia treatment, the net-pen volume was reduced to 54m^2, while the oxygen concentration decreased over the treatment phases from 70.3% in T1, to 53.6% in T2, to 41.9% in T3. The weighted mean depth of the school does not appear to change with phase, from w.m. 1.6m in P, to w.m. 1.8m in T1, w.m. 1.8m in T2, w.m. 1.8m in T3 (Figure 24). Spread of the school (interquartile distance q_5-q_{95}) does not appear to change either, IQR 1.6m in P to IQR 1.2m in T1, IQR 1.4m in T2 and IQR 1.5m in T3. However, the mean volume backscattering coefficient – shown by the horizontal length of the grey box in Figure 24 – increases at the beginning of the treatment (from $1.75 \cdot 10^{-4}$ m^{-2} in the pre-treatment phase P to approximately $3.0 \cdot 10^{-4}$ m^{-2} in treatment phase T1), before decreasing again over the duration of the treatment ($2.0 \cdot 10^{-4}$ m^{-2} in Phase T2 to $1.75 \cdot 10^{-4}$ m^{-2} in Phase T3). These changes in volume backscattering coefficient suggest increases in school density during the treatment.

3.2.2. DENSITY

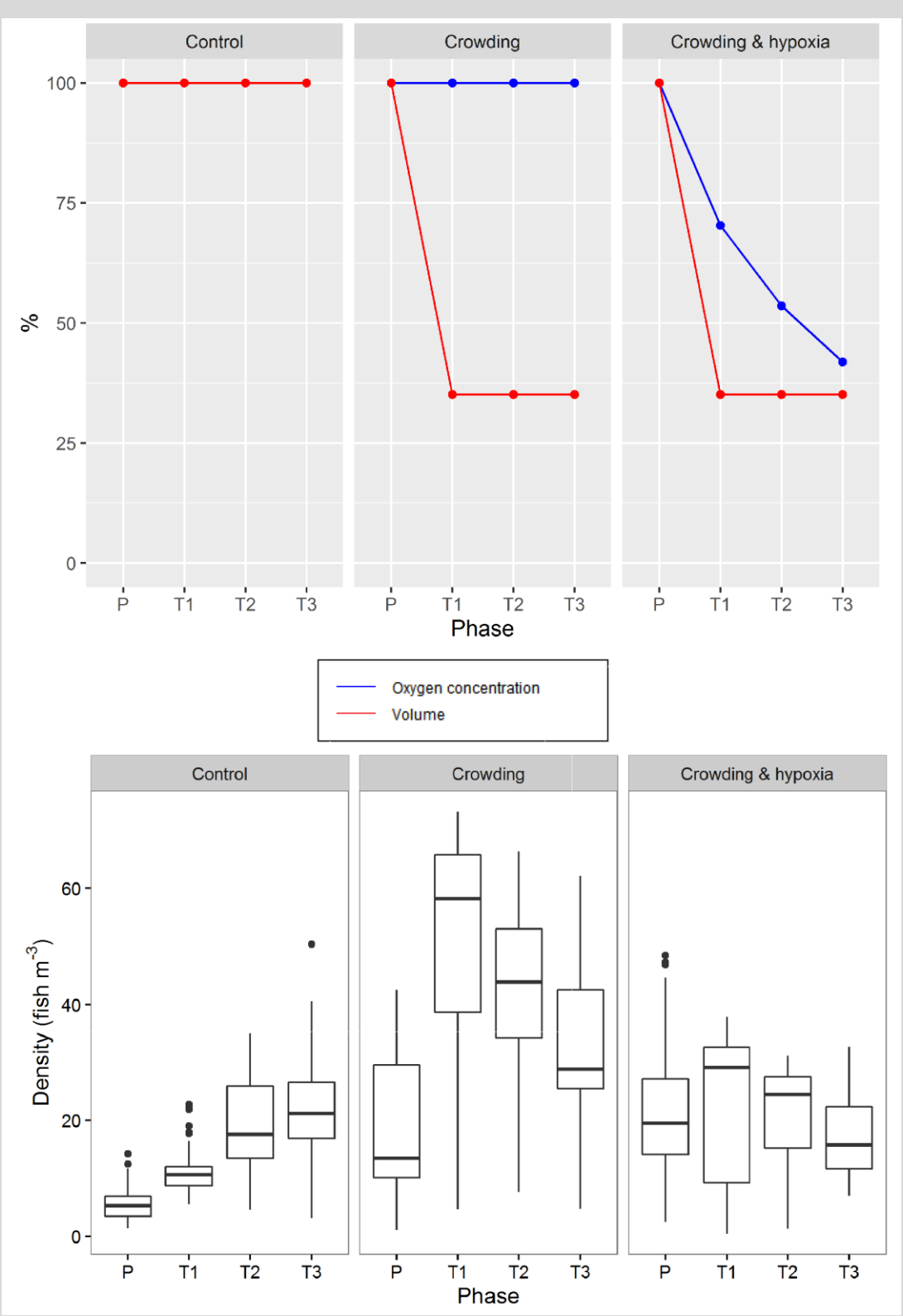

Figure 25. Acoustic estimates of density of the mackerel school (fish per cubic metre) per treatment and phase. P=pre-treatment; T1-3=treatment over time. Each data point represents an estimate of average density per depth layer. Dissolved oxygen concentration (%) and volume of net-pen(%) with phase is plotted above.

The control shows a steady increase over time, from a mean of 5 fish m^{-3} pre-treatment up to 20 fish m^{-3} in the final treatment phase T3.

Density changes in the crowding treatment show a comparatively similar pattern of increase and subsequent decrease as with the crowding and hypoxia treatment. Densities in both treatments increase in Phase T1, before steadily decreasing in Phases T2 and T3. However, the density in the crowding treatment reaches much higher levels (mean density of 60 fish m^{-3}) compared to the crowding and hypoxia treatment (mean density of 30 fish m^{-3}). In addition, the school in the crowding and hypoxia treatment returns to the original density in Phase T3, while the density in the crowding treatment still remains higher at Phase T3 than original pre-treatment densities (mean density of 25 fish m^{-3} compared to 15 fish m^{-3} in pre-treatment phase P).

3.2.3. BIOMASS

The mean weight of all the sampled individual mackerel was 905 grams (±32.17), with a mean length of 39 centimetres (±0.44). Mean values for the control, crowding and crowding & hypoxia net-pens showed biomass estimates of 100kg, 200kg and 125kg respectively (Figure 26).

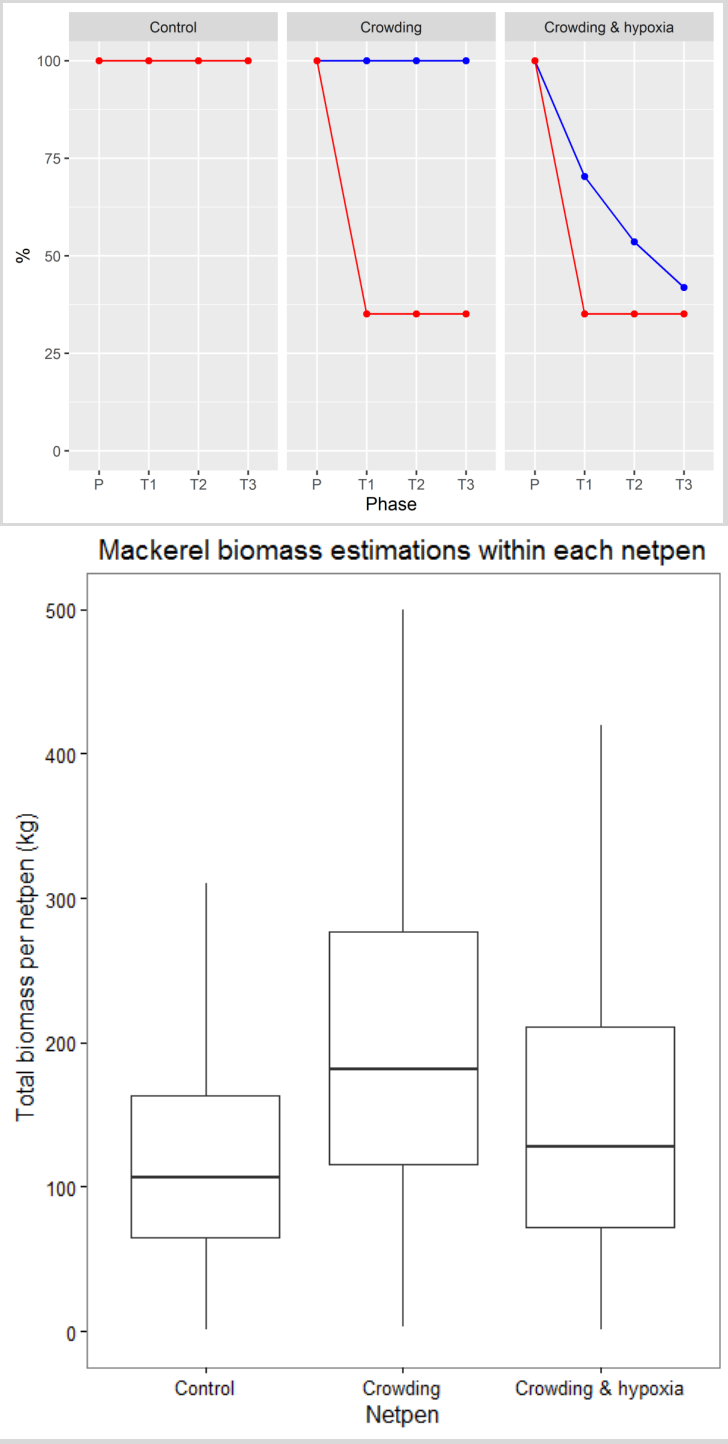

Figure 26. Acoustic estimations of mackerel biomass (kg) per net-pen. Solid black lines in the bars represent the estimated mean biomass, and the spread is a result of using data averaged by each depth layer.

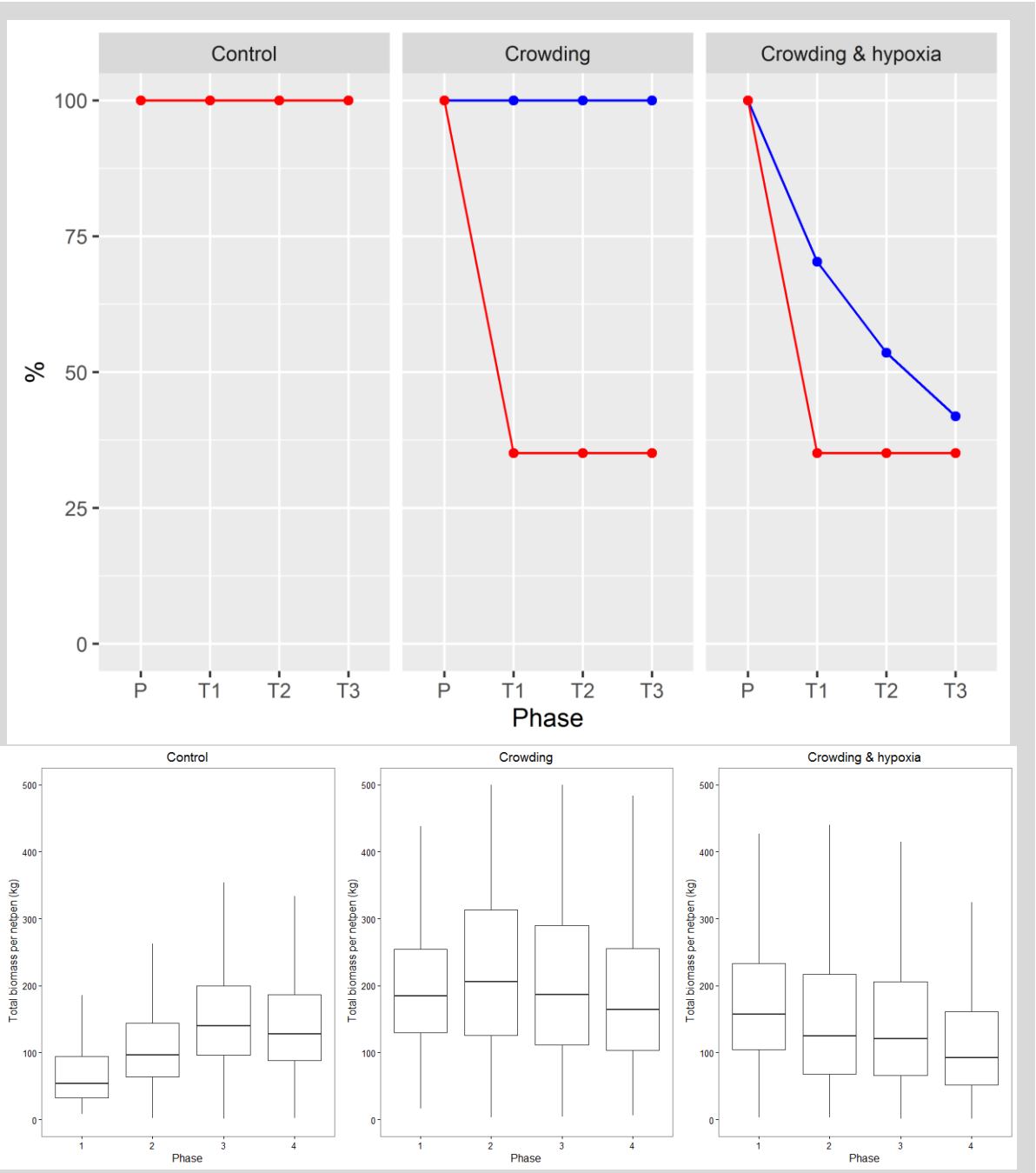

Figure 27. Acoustic estimations of mackerel biomass (kg) per net-pen. Solid black lines in the bars represent the estimated mean biomass, and the spread is a result of using data averaged by each depth layer. % dissolved oxygen concentration (blue) and % volume of net-pen (red) with phase is plotted above.

Biomass estimations appear to increase in the control treatment, remain relatively similar in the crowding treatment, but decrease in the crowding and hypoxia treatment (Figure 27).

The spread of the data shows the between-ping variation of the biomass estimations; the biomass within the netpen did not change throughout the experiments.

4. DISCUSSION

From the visual and acoustic data collected in this thesis, two behavioural metrics have potential for use as behavioural indicators of stress following crowding and/or hypoxia. The first metric is tail beat frequency, collected using visual methods, which shows a significant increase with crowding density and hypoxia. The second metric is density, collected using acoustic methods, which also shows a significant increase with crowding and hypoxia. In all other metrics – tail beat amplitude and vertical distribution – only minor effects of crowding and hypoxia could be observed.

The research objectives will be discussed in further detail below. Individual and school behavioural metrics – including the effects of multiple stressors and potential adaption of mackerel to treatment stress – will be discussed, as well as limitations of the methods and recommendations for future work.

4.1. *Does individual mackerel swimming activity change with crowding and hypoxia?*

4.1.1. TAIL BEAT FREQUENCY

Tail beat frequency showed significant increases compared to the pre-treatment control phase when crowded in the purse seine simulations.

Mackerel increase activity with crowding, which could be an adaptive stress response to increase the possibility of survival in suboptimal environments (Barton & Iwama, 1991; Johnson et al., 1992; Schreck et al., 1997; Pottinger, 2008). Typically, higher activity levels are associated with anti-predatory responses or avoidance of perceived threats (in this case, the simulated 'capture' in the net-pen; Domenici et al., 2007; Domenici, 2010; Marras & Domenici, 2013). It could also be possible that individuals within the school are repositioning, in order to seek more protection from the perceived threat (Magurran et al., 1993; Romey, 1996).

During the treatment phases, tail beat frequency did not differ significantly from phase to phase in any of the treatments. It seems that the increase in tail beat frequency is only a short-term response –prolonged exposure (over one hour) to a stressor normally results in a more intense stress response i.e. that tail beat frequency would keep increasing with exposure to crowding and hypoxic conditions (Lockwood et al., 1983). There could be several reasons for these findings. Either the stressors were not strong enough to elicit any long-term behavioural

responses during the treatment phases, with the mackerel showing an adaptive behavioural response to the treatment, or there were underlying physiological stress responses that did not manifest itself through these particular behavioural metrics. Another possibility could be that under increasing intensity of the stressor/s, a new coping strategy of lower tail beat frequencies was used in order to avoid physiological exhaustion (He, 1993; Domenici et al., 2000; Broadhurst, 2006). Faster rates of recovery to original tail beat frequencies are seen in the crowding treatment than in the crowding and hypoxia treatment. This could be indicative of longer lasting stress responses with hypoxia in the monitoring phases, especially as in the later monitoring phases.

Despite the additional hypoxia stressor, the mean tail beat frequencies during the crowding and hypoxia treatment did not differ significantly from the crowding treatment. This means that multiple stressors did not have an additive effect on tail beat frequency behaviour. This could be demonstrative of an interaction between multiple stressors (Crain et al., 2009). In this case, the addition of the hypoxia stressor appears to show an antagonistic effect on tail beat frequency, here used to define a cumulative effect that is less than additive (Folt et al., 1999; Piggott et al., 2015).

There could be multiple reasons why there is no significant difference between the treatments. There could be a behavioural trade-off between inter-fish distance and respiratory distress; the need for more oxygen in hypoxic conditions and the need to crowd closer to maintain school structure and avoid predation (Domenici et al., 2000). This trade-off could be stronger in fish that require higher dissolved oxygen concentrations to avoid oxidative stress at sustained high-speed swimming behaviour, such as mackerel and sharks (Carlson & Parsons, 2001). Both the crowding and crowding & hypoxia treatments showed initial increases in tail beat frequency. In the crowding treatment, this may have been due to a fright response, reaching higher activity levels overall. In the crowding & hypoxia treatment, this might indicate an attempt to move away from the hypoxic zone, but eventually reduce the activity levels to reduce energetic (and oxygen) demands. The lower activity levels might have been an adaptive response by the mackerel to conserve energy in order to delay exhaustion, which has been suggested to be a major source of mortality in herring and sardine purse seine fisheries (Domenici et al., 2000; Marçalo et al., 2013; Morgan, 2014).

Another reason could relate to the physiological adaptations of mackerel to low environmental oxygen concentrations. Mackerel are active pelagic teleost fish with an enhanced blood oxygen capacity, a condition enabling them to cope with increased oxygen demands (Filho et al., 1992). Swift (1982) found that mackerel are capable of increasing blood haemoglobin concentrations

following confinement stress (13.4g/100ml in stressed mackerel vs. 10.3g/100ml in unstressed mackerel). This stress-induced increase in haemoglobin concentration allows increased oxygen carrying capacity from the gills and to the muscles (Swift 1983), and could provide a hypothetical reason why the addition of hypoxia stressors do not show a significant change in tail beat frequencies compared to crowding alone.

Interaction between multiple stressors may not necessarily be additive with respect to behavioural responses. This needs to be taken into consideration when studying fish behaviour that involves multiple stressors, particularly when utilising RAMP vitality assessment – a weaker behavioural response does not necessarily mean that the fish are unstressed (Davis 2010, Raby et al. 2012).

4.1.2. TAIL BEAT AMPLITUDE

Tail beat amplitude was found not to differ significantly between treatments or phases, and so shows no evidence of adaption or recovery.

There could be several reasons for this.

Firstly, mackerel has a streamlined body shape typical of fast swimming fish (Videler & Hess, 1984; Sfakiotakis et al., 1999). As a highly migratory species, mackerel are very efficient swimmers, capable of swimming very long distances at a sustainable speed. As a result, the tail beat amplitude varies little throughout the tail beat cycle in order to maintain a steady speed (Akanyeti et al., 2016).

Also, the mackerel were contained inside a net-pen, where they needed to swim in a constantly turning circle to keep schooling in an elliptical shape (Brierley & Cox, 2010). This would involve one tail beat with a large amplitude for the main turn motion, before a much smaller half-tail beat to steady the rest of the body after the turn was completed. Swimming in circles also introduces an extra element of centripetal force requiring more energy for continuous motion (Weihs, 1981).

Finally, measurement errors could have made very small differences to tail beat amplitude. As body length and tail beat measurements were taken manually in ImageJ software (Figure 17 in Methods), it is possible that observer error might have occurred. However, differences would be minute – as length was measured in pixels, an error of a few pixels would equate to a few millimetres of measurement error, depending on the distance of the fish from the vertical camera. Overall, it is unlikely that this would cause serious changes in the results.

4.1.3. EXPERIMENTAL CRITIQUE OF VISUAL METHODS

During treatment phases T1 to T3 in the crowded net-pens, the vertical camera was lifted up along with the base of the net-pen. This led to the camera being positioned close to fast swimming mackerel (Figure 28), and an individual mackerel was in the field of view for less than a second. If possible, it would be more suitable to keep the vertical camera below the school during crowding, without intruding into the school and causing fish to swim around the obstacle. This could be done by positioning the camera in a corner, before sweeping it below the school into the centre, or by keeping the camera at a lower depth.

Figure 28. Example of mackerel video footage when vertical camera was raised during the crowding treatment.

Poor weather conditions could limit video footage resolution. Although vertical camera footage was clear at Austevoll Research Station due to good weather conditions and shallow water, such good lighting and strong contrast might not be available in larger experimental seapens, or in a commercial purse seine environment, particularly at night. In such cases, other visual methods such as infrared cameras might be required to observe the fish without disturbance (as fish cannot perceive infrared; Widder et al., 2005). However, with a limited range (1m in Rose et al., 2005) due to the high attenuation in seawater, this would limit the potential field-of-view to outer individuals in the school (Pegau et al., 1997; Chidami et al., 2007).

Different light intensities might also affect individual swimming behaviour. As vision is the predominant sensory system for schools (Partridge & Pitcher, 1980; Miyazaki et al., 2000), low light conditions could affect the mackerel ability to school by reducing the fish visual acuity and ability to orient to neighbours (McMahon & Holanov, 1995; Miyazaki et al., 2000; Ryer & Olla, 2000; Domenici et al., 2002). In some species, fish swimming speed decreases when light

intensity decreases, as it is more difficult to transfer information across the school and maintain parallel orientation at higher speeds (Walsh & Hickey, 1993; Katz et al., 2011). In future research, some effort must be made to account for ambient light conditions and weather, using an okta system or measuring light levels.

The GoPro vertical cameras were only capable of up to 2 hours of continuous recording at high resolution before running out of battery. It was impossible to remove the camera and replace the battery without disturbing the school and disrupting fish behaviour. This led to missing data for Phase T3 of the crowding and hypoxia treatment, which could have provided more insight into the duration effects on tail beat frequency and amplitude. While the GoPro vertical camera was appropriate for short recordings (such as during the post-stress monitoring phases), alternative cameras with a constant power source might be more appropriate for recording the main experiment. Incorporating automation into the processing of behavioural metrics in the video footage – particularly of tail beat frequency – would be helpful, if possible. Real-time imaging-processing software would provide a valuable asset in automated analysis (Morgan, 2014), as it would provide an immediate view of behaviour at that time and under those conditions. This could also reduce the likelihood of missing data through camera malfunctions and battery outages.

4.2. *Does mackerel school structure and distribution change with crowding and hypoxia?*

4.2.1. VERTICAL DISTRIBUTION

Changes in vertical distribution of the school were observed between treatments and phases, at the scale of up to 1 metre, which may be significant in this shallow environment. At the start of the treatment, mackerel appeared to swim upwards towards the surface. The schools in the control net-pen and the crowding treatment appeared to move higher up in the water column over the phases, while the school in the crowding and hypoxia treatment remained relatively high in the water column throughout the experiment. This is unusual, as in most fish species, exposure to negative stimuli leads to rapid escape movements towards the bottom of the tank or cage, with concentrations near the bottom indicating relatively recent exposure to acute stressors (Stien et al., 2007; Martins et al., 2012). However, some fish species such as haddock *Melanogrammus aeglefinus* have demonstrated upward escape responses from approaching gear during capture (Main & Sangster, 1982; He, 1993), and it is possible that mackerel could demonstrate a similar escape response from the purse-seine net.

The spread of the school was narrower in the control and crowding treatments in Phases T1-T3 compared to the crowding and hypoxia treatment phases T1-T3, also reflected in the density changes. This could show a potential stress response to crowding, but antagonistic effects on responses with the addition of hypoxia.

In the crowding and hypoxia treatment, there could have been underlying physiological mechanisms behind these findings that may not be detectable in the data. In a hypoxic environment, fish tend to swim up to the surface of the water column where there is a relatively higher concentration of dissolved oxygen via diffusion at the air-surface interface. This behaviour is known as aquatic surface respiration or ASR (Kramer, 1987; Reebs, 1994). However, the school in the crowding and hypoxia treatment maintained a greater depth range compared to the other treatments, suggesting that they did the opposite of swimming closer to the surface, and directly contradicts the ASR hypothesis. Typically, fish in a purse seine tend to swim downwards as an escape response (Misund, 1993), so multiple pressures could be disguised as a lack of response to a crowded net-pen.

The changes in the spread of the school in the control group with phase could infer density increases over time, despite the absence of stressors. These findings could be due to random changes, as the fish had more space to occupy within the net-pen, or other external factors (such as avoidance of observational equipment, or cumulative stress responses to the predator-avoidance tests).

4.2.2. DENSITY

Mackerel in the crowding treatment reached much denser concentrations than the control treatment. This suggests that the reduction of net-pen volume forced the mackerel closer together, and the adoption of a more defensive school formation. Denser schools allow the transfer of information between individuals to occur quicker with smaller inter-fish distances, allowing faster responses for avoidance of perceived threats. Reducing the net-pen volume limited the space that the mackerel individuals could inhabit, therefore changing the school density without any vertical movement upwards in the water column.

The inclusion of hypoxia did not have an additive effect on mackerel density. In fact, hypoxia reduces the effects of crowding on mackerel density, acting as an antagonistic effect. Schooling behaviour in fish follows certain individual-based rules – attraction between fish, repulsion from other fish and foreign objects, and alignment to neighbours (Pitcher & Parrish, 1993; Gueron et al., 1996; Parrish et al., 2002; Tien et al., 2004). As in the tail beat frequency results, this could reflect a behavioural trade-off – this time, between the need to maintain schooling

behaviour (the attraction rule), and the lower oxygen concentrations per volume of water. Increasing inter-fish distances results in fewer fish per volume, and so less consumed oxygen per volume. This makes the spacing out of individuals advantageous to maximize the use of the limited oxygen availability.

School densities also demonstrated quick adaption to the treatments, peaking in the T1 phase before decreasing to the original densities by T3. Changes in density in the control occurred over time, although it was in the later phases of the treatment. As this behavioural change was not associated with experimental stressors, it is assumed that this is randomly occurring. Recovery rates from the treatment effect could be observed by improving the resolution of observations immediately after the stressors. In this study, mackerel were found to have returned to their original tail beat frequencies within 20 hours after the treatment, suggesting that high recovery rates were seen in these mackerel.

Although fish densities remained consistently lower in the control net-pen than in the other treatment net-pens, the control mackerel showed an increasing density over time despite a lack of experimental stressors. This could have been due to a number of unaccounted random effects. For instance, strong currents could have altered fish behaviour on the day of the experiment (Castonguay and Gilbert, 1995) and the predator avoidance experiments conducted as part of the experimental protocols could also have contributed to mackerel stress responses over time.

4.2.3. BIOMASS

Mackerel biomasses in the net-pens were intentionally kept low to avoid chronic stress leading to mortality. The mackerel biomass present in the crowding and hypoxia net-pen was lower compared to the crowding net-pen. However, these relative differences in biomass between the net-pens (Figure 26) are not likely to be large enough to show much difference in 'normal' unstressed fish behaviour.

4.2.4. EXPERIMENTAL CRITIQUE OF ACOUSTIC METHODS

One of the main issues with the acoustic data collection was the lack of replicates in the data. As there was only one replicate for each treatment, it is not possible to carry out statistical analysis on the results, and so the acoustic data can only be treated as a descriptive snapshot of what is happening to school distribution and density over that set period of time. For the purposes of this thesis, this should be acceptable as an overview of the main patterns and changes with treatment over time, but for further research, more replicates may be needed for statistical analysis.

Limited coverage of the net-pen by the acoustic beam could also have caused discrepancies in the acoustic data. The EK60 echosounder was placed in the centre of the net-pen floor, looking vertically upwards towards the surface. If there is a vacuole in the centre of the school (Figure 29), there is a high chance that the acoustic beam may miss a portion of the school. This effect could have been exacerbated further by horizontal movement of the fish – the use of a narrow beam (opening angle of 7°) does not cover much area or volume of the school, especially as it is close to the surface (approximately 5 to 6.5 metres depth). Fish closer to the echosounder could also be missed due to being within the acoustic near-field deadzone, where acoustic data were excluded due to noise.

Figure 29. Vacuole formation in the centre of a school can be a limitation in acoustic methods using narrow beam widths.

Uncertainty in target strength of the mackerel could also have biased acoustic data. Target strength of an individual fish depends on many different factors, such as the fish size, physiology and swimming behaviour, particularly swim tilting angle (Huse & Ona, 1996; Georgakarakos et al., 2011). Mackerel used in these experiments were very large compared to wild populations, and the fat content could be much higher than wild fish (Cook et al., 2000), which could affect target strength (Ona, 1990). Negative buoyancy could affect the swim tilt angle of mackerel (Korneliussen & Ona, 2002), providing less area for detection by the acoustic beam and causing further bias.

Mackerel behaviour was only observed during daylight hours during the experiments. This could have unintentionally increased the stability of schooling compared to night-time hours, or during adverse weather conditions. Various studies have found fish schools to behave

differently in the day compared to at night – during low light levels, mackerel are more likely to swim as individuals and the distance between individuals is increased to avoid collisions with other fish, therefore reducing the school density (Glass et al., 1986; Olla et al., 2000; Domenici et al., 2002).

4.3. *Are the behavioural metrics used in this thesis useful stress indicators for welfare in purse seine fisheries?*

Poor welfare is associated with overtaxing the coping capacity of animals, known as an allostatic overload (McEwen & Wingfield, 2003; Boerrigter et al., 2015). The welfare state of the fish will affect the schooling rules of attraction, alignment and repulsion used by the fish (Barton, 2002; Sih et al., 2004; Huntingford et al., 2006). Model studies have shown that only one fish with different schooling rules can affect the behaviour of the whole school (Romey, 1996), indicating that individual behaviour can be a sensitive indicator.

The FAO code of conduct for responsible fisheries (FAO, 1995; Garcia, 2000) suggests that states should carry out studies on the behaviour of target and non-target species in relation to fishing gear as an aid for management decisions, and with a view to minimizing unwanted catches. Behavioural metrics provide a reliable and less invasive method of welfare assessment on fish than physiological methods, which require capture and handling (Dawkins, 2004; Ashley, 2007; Martins et al., 2012). Behavioural stress responses are more immediately apparent than physiological stress responses, and in a large-scale commercial purse-seine fishery, these changes are more easily monitored and are far less costly to detect than through physiological methods.

From the behavioural metrics studied in this thesis, tail beat frequency and school densities have the most potential to be useful welfare indicators in commercial purse seine fisheries. Both of these behavioural metrics showed the greatest changes when exposed to crowding and hypoxia stressors, and subsequent faster rates of post-treatment recovery. This suggests that mackerel slipped from a purse seine under these conditions have a very high likelihood of post-release survival.

4.4. FUTURE RECOMMENDATIONS

In order to better understand the synergistic versus separate effects of crowding and hypoxia, an extra net-pen with only hypoxic treatment could be used for further analysis on mackerel behaviour. It is important to know the behavioural responses of mackerel with only hypoxia as a stressor, especially as mackerel has a high oxygen demand as a highly active swimming fish (Massabau 2001, 2003; Domenici et al., 2007). The density could be even more affected by fish that require high concentrations of oxygen (a much larger volume of water) surrounding the gills with higher swimming activity (Carlson & Parsons, 2001). This must be taken into consideration when observing fish behaviour with multiple stressors – hypoxic effects could negate the effects of crowding, and could mask the observable behavioural stress response.

While we measured schools with biomasses between approximately 125kg and 200kg in the net-pens, fish captured in commercial purse seines represent much greater biomasses (Huse & Kvamme (2008) reported mackerel purse seine landings from individual vessels in the region of 12 to 37 tonnes in 2006). Higher biomasses of fish are recommended than was used in the net-pens during this experiment for future research, as this would better simulate a commercial purse seine haul. Better methods of controlling how many fish are in each net-pen are needed, possibly by observing transferred fish with acoustics as well as through visual estimations.

Using a higher temporal resolution of video collection post-treatment would gain more information on post-stressor behaviour. Observations every hour, or even every 30 minutes, would provide a better understanding of how behaviour changes immediately following the stressor treatments. This would be more informative about post-release behaviour of mackerel from a purse seine, at a time when fish would be most vulnerable to predation from animals not subjected to the same crowded and hypoxic conditions, such as seabirds, and marine mammals including seals and cetaceans (Ryer, 2004; Zhou et al., 2007).

These experiments were carried out on mackerel that had been kept in captivity for over a year in an aquaculture net-pen and fed daily on aquaculture pellets. As a result, the individuals observed in this experiment were very large (means of 905g and 39cm), which is much larger than typical wild populations. Fish that have been more recently captured should be used in future experiments, as the capacity of fish to respond to acute stressors may be altered by habituation to a captive environment (Barton et al., 2005). Larger fish, such as the individuals used in these experiments, are more resilient to stress and are more likely to survive than smaller individuals (Olsen et al. 2012). The diet of captive mackerel should be better controlled to reflect that of wild populations, particularly as increased hunger in individuals loosens school

structure by increasing individual food-search behaviour (Robinson & Pitcher, 1989a,b). This can affect fish density and school cohesiveness. Appetite and digestion of food could be affected by post-treatment responses to the original treatment stress, and this might have an impact on long-term survival of mackerel post-slipping (Temming et al., 2002).

The size of the individual mackerel could also have a positive impact on the schooling cohesion – fish of larger sizes are found to recognize each other at longer maximum distances compared to smaller individuals, with a strong correlation found between eye size and light intensity threshold (Higgs & Fuiman, 1996; Bilotta, 2000). How mackerel respond in light or dark conditions could have a strong impact on commercial purse seine fisheries, particularly during night fishing, when fish schooling behaviour could be disrupted, more collisions between individuals and with the purse-seine net, and potentially increased mortality could occur (Cui et al., 1991; Huse & Vold, 2010). Because of this, more experiments on school structure might be needed during night-time (or at least, simulated low light levels) to find out how light affects density and activity levels of schools. This will also maintain an equal light intensity between experimental days, not influenced by changing weather conditions.

4.5. CONCLUDING REMARKS

Of the metrics observed in this thesis, tail beat frequency and fish densities are the best potential stress indicators for welfare in mackerel during purse seine fishing. Using both visual and acoustic methods provides a clearer picture of both school and individual behaviour. Mackerel show behavioural stress responses – increased tail beat frequency and density – even at non-lethal stressor levels. This is promising, and further extensions to this research – such as inclusion of stereocameras, and increasing the range of stressors – could provide even more detail as to the changes in behavioural stress responses comparable to that of wild populations in a commercial purse seine. This may help to improve current regulations on slipping, which currently state that it is illegal to release captured fish that appear to be dead or dying (Fiskeridirektoratet, 2004.) Behavioural indicators will allow easier detection of over-stressed fish, and can allow fishers to adhere to these rules of slipping, and improve management of the slipping process in commercial purse seine fisheries.

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APPENDICES

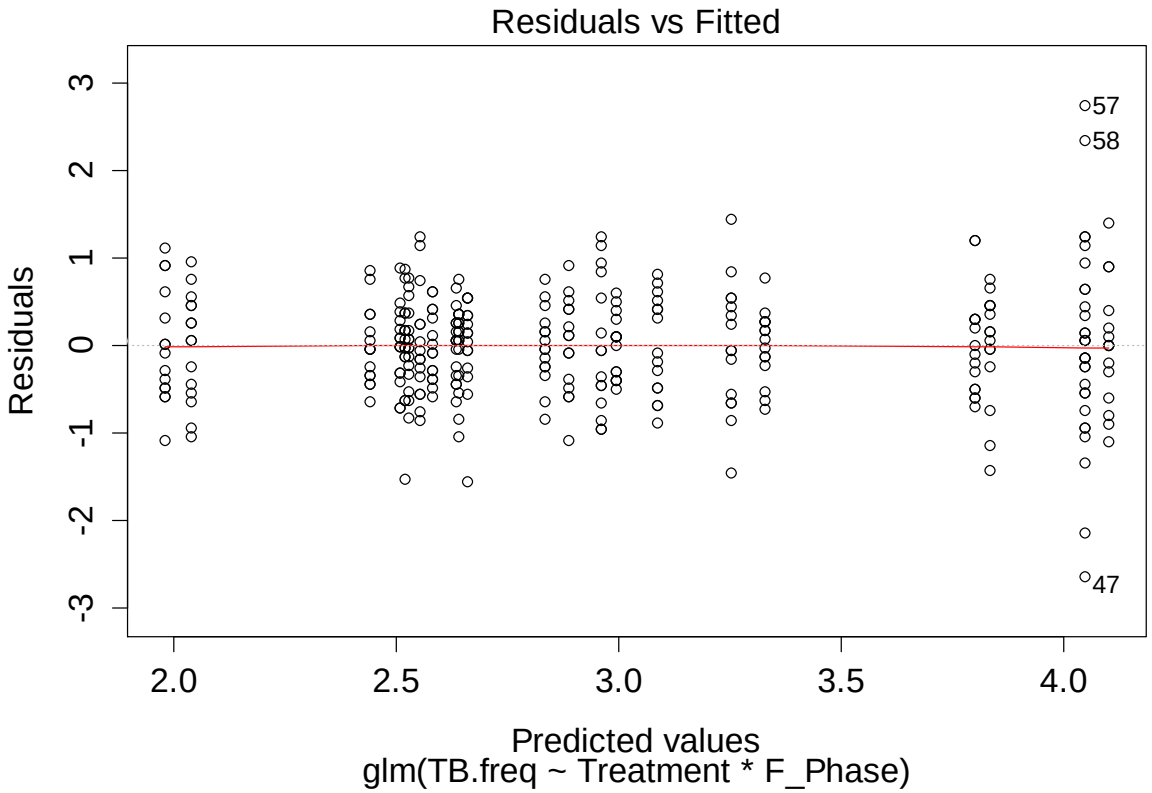
APPENDIX 1

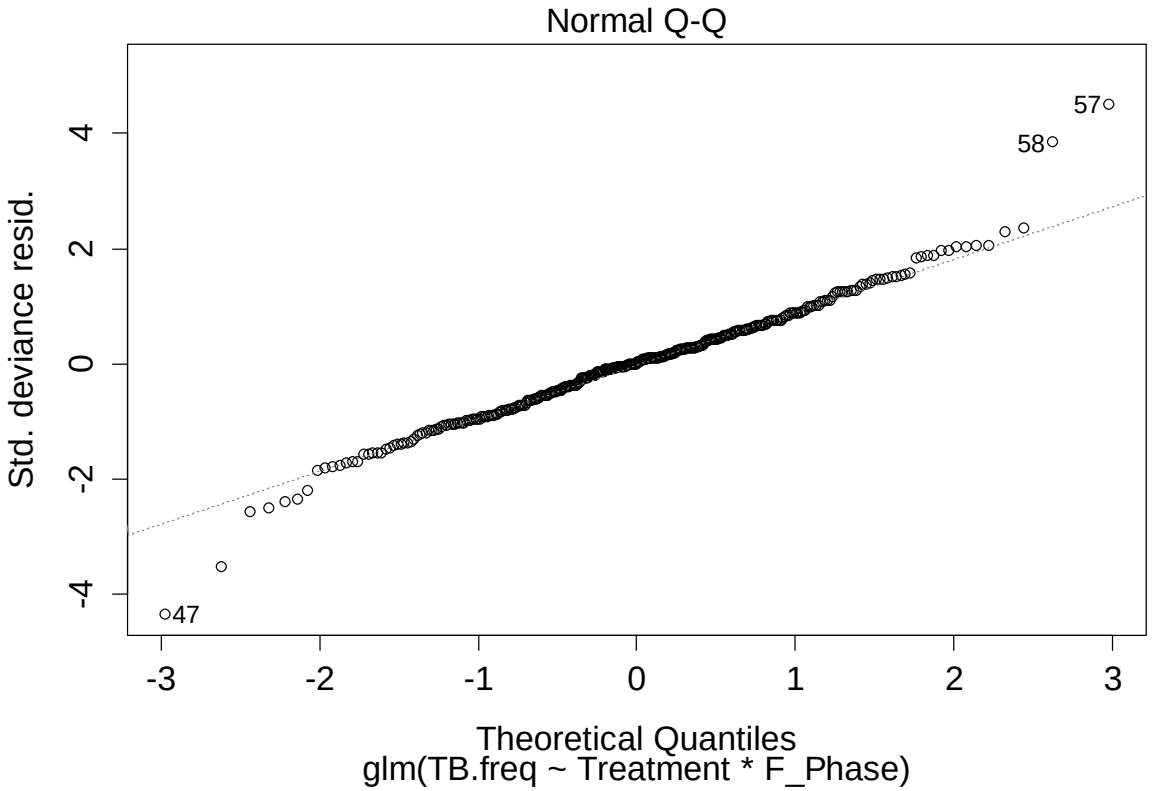
Netpen	Treatment	Phase	Clip.no	Time of clip (GMT)	Time since treatment start (hours)	Volume (m3)	Oxygen (%)
1	Crowding & hypoxia	P	0044	09:00:00	-1	154.17	100
1	Crowding & hypoxia	P	0045	08:57:39	-1	154.17	100
1	Crowding & hypoxia	P	0046	09:34:02	-0.5	154.17	100
1	Crowding & hypoxia	T1	0050	10:16:28	0.25	54.17	71
1	Crowding & hypoxia	T1	0051	10:17:53	0.25	54.17	71
1	Crowding & hypoxia	T1	0052	10:14:39	0.25	54.17	71
1	Crowding & hypoxia	T2	0056	11:59:16	2	54.17	51
1	Crowding & hypoxia	T2	0057	12:01:14	2	54.17	51
1	Crowding & hypoxia	T2	0058	11:47:33	1.75	54.17	53
1	Crowding & hypoxia	M1	0062	14:53:00	28.75	154.17	100
1	Crowding & hypoxia	M1	0063	14:44:01	28.75	154.17	100
1	Crowding & hypoxia	M1	0064	14:51:57	28.75	154.17	100
1	Crowding & hypoxia	M2	0068	09:10:34	47	154.17	100
1	Crowding & hypoxia	M2	0069	09:11:43	47	154.17	100
1	Crowding & hypoxia	M2	0070	09:05:46	47	154.17	100
1	Crowding & hypoxia	M3	0074	08:51:41	70.75	154.17	100
1	Crowding & hypoxia	M3	0075	08:38:05	70.75	154.17	100
1	Crowding & hypoxia	M3	0076	08:39:29	70.75	154.17	100
1	Crowding & hypoxia	M4	0080	08:03:27	142	154.17	100
1	Crowding & hypoxia	M4	0081	08:05:15	142	154.17	100
1	Crowding & hypoxia	M4	0082	08:04:34	142	154.17	100
2	Crowding	P	0092	09:05:34	-2.5	154.17	99
2	Crowding	P	0093	08:31:32	-2.5	154.17	99
2	Crowding	P	0094	08:34:30	-2.5	154.17	99
2	Crowding	T1	0098	11:06:54	0	54.17	100
2	Crowding	T1	0099	11:14:23	0.25	54.17	100
2	Crowding	T1	0100	11:06:43	0	54.17	100
2	Crowding	T2	0104	12:25:31	1.5	54.17	100
2	Crowding	T2	0105	12:16:15	1.25	54.17	100
2	Crowding	T2	0106	12:21:24	1.5	54.17	100
2	Crowding	T3	0110	13:07:11	2	54.17	100
2	Crowding	T3	0111	13:05:59	2	54.17	100
2	Crowding	T3	0112	13:03:34	2	54.17	100
2	Crowding	M1	0116	13:26:39	26.5	154.17	100
2	Crowding	M1	0117	13:38:19	26.5	154.17	100
2	Crowding	M1	0118	13:23:42	26.5	154.17	100
2	Crowding	M2	0122	07:49:12	44.75	154.17	100
2	Crowding	M2	0123	07:44:31	44.75	154.17	100
2	Crowding	M2	0124	07:43:27	44.75	154.17	100
2	Crowding	M3	0128	09:24:40	70.5	154.17	100
2	Crowding	M3	0129	09:32:56	70.5	154.17	100
2	Crowding	M3	0130	09:26:05	70.5	154.17	100
2	Crowding	M4	0134	10:42:48	143.75	154.17	100
2	Crowding	M4	0135	10:41:22	143.75	154.17	100
2	Crowding	M4	0136	10:43:47	143.75	154.17	100
3	Control	P	0140	08:14:31	-0.75	154.17	100
3	Control	P	0141	08:07:52	-1	154.17	100
3	Control	P	0142	08:13:14	-0.75	154.17	100
3	Control	T1	0146	09:30:52	0.5	154.17	100
3	Control	T1	0147	09:35:36	0.5	154.17	100
3	Control	T1	0148	09:39:04	0.75	154.17	100
3	Control	T2	0152	11:02:37	2	154.17	100
3	Control	T2	0153	11:02:02	2	154.17	100
3	Control	T2	0154	11:10:25	2.15	154.17	100
3	Control	T3	0158	12:11:32	3.15	154.17	100
3	Control	T3	0159	12:06:59	3	154.17	100
3	Control	T3	0160	12:18:35	3.15	154.17	100
3	Control	M1	0164	08:15:21	23.25	154.17	100
3	Control	M1	0165	08:22:10	23.25	154.17	100
3	Control	M1	0166	08:16:23	23.25	154.17	100
3	Control	M2	0170	10:00:55	49	154.17	100
3	Control	M2	0171	09:59:21	49	154.17	100
3	Control	M2	0172	09:58:00	49	154.17	100
3	Control	M3	0176	07:52:53	70.75	154.17	100
3	Control	M3	0177	07:55:58	71	154.17	100
3	Control	M3	0178	07:49:24	70.75	154.17	100
3	Control	M4	0182	07:45:31	142.75	154.17	100
3	Control	M4	0183	07:47:29	142.75	154.17	100
3	Control	M4	0184	07:52:13	142.75	154.17	100

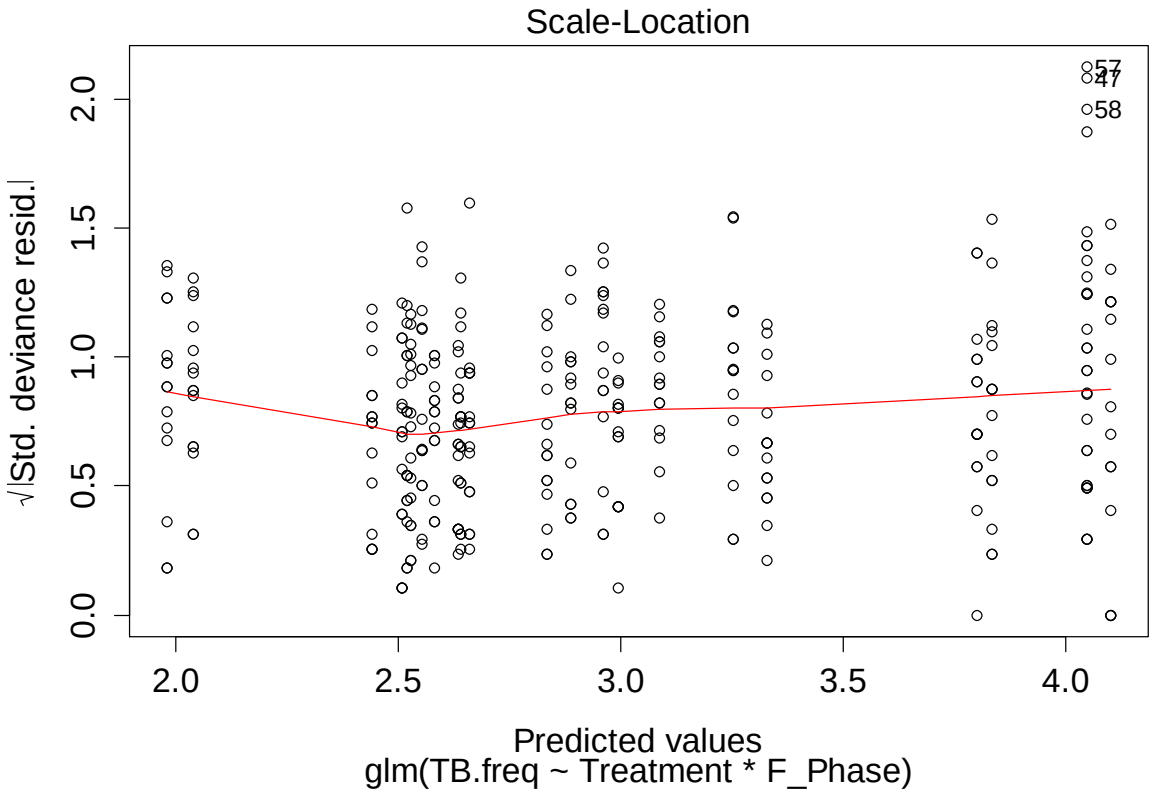
APPENDIX 2

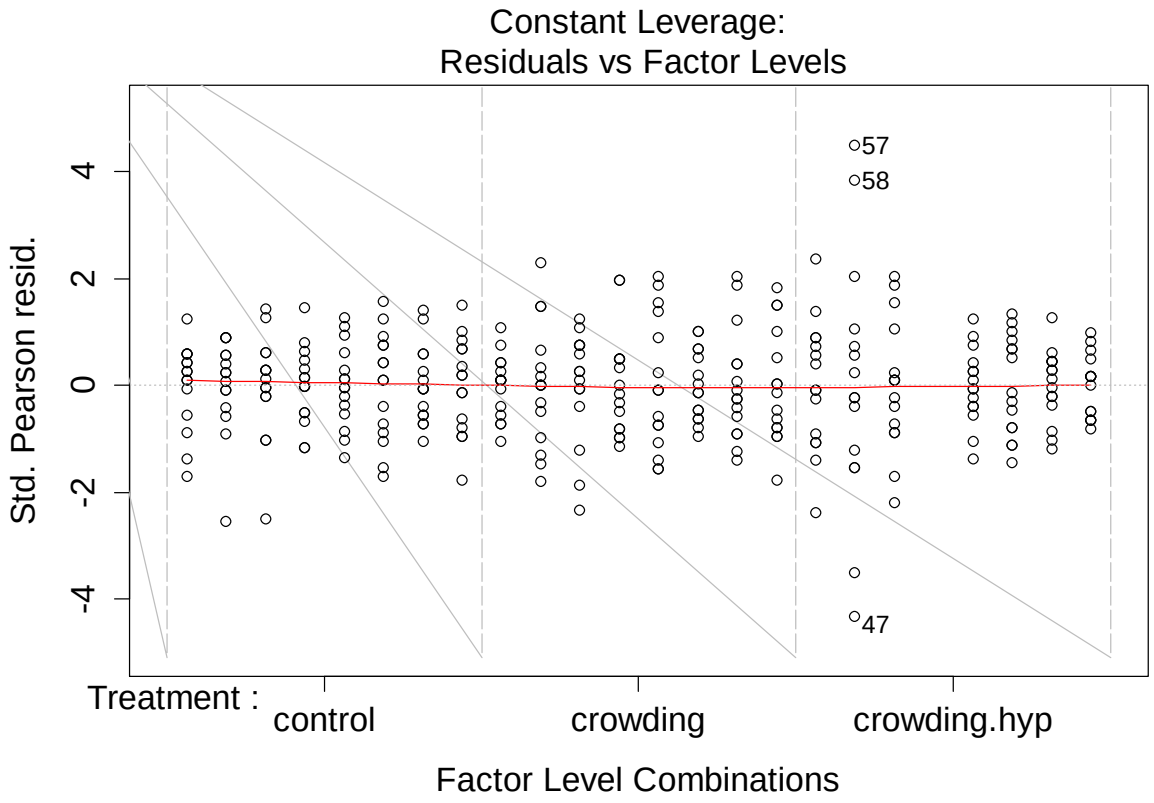
MODEL FITTING: Generalized linear model with Gaussian (normal) distribution

```
GLM with Gaussian ("normal") Distribution ###
>
> GLM_3 <- glm(TB.freq~Treatment*F_Phase, family = gaussian(link =
"identity"), data= fish.df)
> summary(GLM_3)

Call:
glm(formula = TB.freq ~ Treatment * F_Phase, family = gaussian(link = "identity"),
    data = fish.df)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-2.64667  -0.39333   0.00667   0.36000   2.75333

Coefficients: (1 not defined because of singularities)
                                Estimate Std. Error t value Pr(>|t|)
(Intercept)                     2.640000   0.163333  16.163  < 2e-16 ***
Treatmentcrowding              -0.006667   0.230988  -0.029  0.97699
Treatmentcrowding.hyp           0.613333   0.230988   2.655  0.00832 **
F_PhaseE1                       0.020000   0.230988   0.087  0.93106
F_PhaseE2                      -0.120000   0.230988  -0.520  0.60376
F_PhaseE3                      -0.133333   0.230988  -0.577  0.56419
F_PhaseM1                      -0.113333   0.230988  -0.491  0.62401
F_PhaseM2                      -0.600000   0.230988  -2.598  0.00982 **
F_PhaseM3                      -0.200000   0.230988  -0.866  0.38722
F_PhaseM4                       0.246667   0.230988   1.068  0.28638
Treatmentcrowding:F_PhaseE1     1.446667   0.326666   4.429 1.30e-05 ***
Treatmentcrowding.hyp:F_PhaseE1 0.773333   0.326666   2.367  0.01851 *
Treatmentcrowding:F_PhaseE2     1.320000   0.326666   4.041 6.67e-05 ***
Treatmentcrowding.hyp:F_PhaseE2 0.913333   0.326666   2.796  0.00549 **
Treatmentcrowding:F_PhaseE3     1.300000   0.326666   3.980 8.53e-05 ***
Treatmentcrowding.hyp:F_PhaseE3       NA         NA      NA       NA
Treatmentcrowding:F_PhaseM1     0.440000   0.326666   1.347  0.17895
Treatmentcrowding.hyp:F_PhaseM1 -0.306667  0.326666  -0.939  0.34855
Treatmentcrowding:F_PhaseM2     0.546667   0.326666   1.673  0.09521 .
Treatmentcrowding.hyp:F_PhaseM2 0.433333   0.326666   1.327  0.18560
Treatmentcrowding:F_PhaseM3     0.120000   0.326666   0.367  0.71360
Treatmentcrowding.hyp:F_PhaseM3 0.273333   0.326666   0.837  0.40336
Treatmentcrowding:F_PhaseM4    -0.900000   0.326666  -2.755  0.00620 **
Treatmentcrowding.hyp:F_PhaseM4 -0.506667  0.326666  -1.551  0.12188
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for gaussian family taken to be 0.4001656)

    Null deviance: 258.76  on 344  degrees of freedom
Residual deviance: 128.85  on 322  degrees of freedom
AIC: 687.29

Number of Fisher Scoring iterations: 2

> anova(GLM_3, test = "F")
Analysis of Deviance Table

Model: gaussian, link: identity

Response: TB.freq

Terms added sequentially (first to last)


                 Df Deviance Resid. Df Resid. Dev       F    Pr(>F)
NULL                              344     258.76
Treatment         2   41.121       342     217.64 51.3802 < 2.2e-16 ***
F_Phase           7   50.418       335     167.22 17.9991 < 2.2e-16 ***
Treatment:F_Phase 13  38.367       322     128.85  7.3752 1.088e-12 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

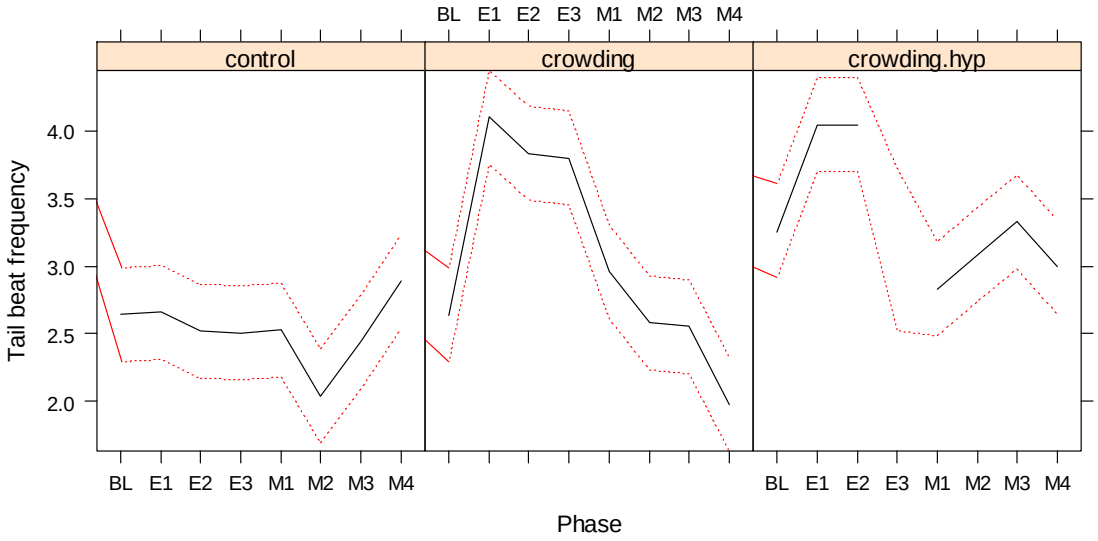

Residuals vs Fitted
Residuals
Predicted values
glm(TB.freq ~ Treatment * F_Phase)
57
58
47


Normal Q-Q
Std. deviance resid.
Theoretical Quantiles
glm(TB.freq ~ Treatment * F_Phase)
57
58
47


Scale-Location
√|Std. deviance resid.|
0.0
0.5
1.0
1.5
2.0
57
47
58
2.0
2.5
3.0
3.5
4.0
Predicted values
glm(TB.freq ~ Treatment * F_Phase)


Constant Leverage:
Residuals vs Factor Levels
Std. Pearson resid.
-4
-2
0
2
4
57
58
47
Treatment :
control
crowding
crowding.hyp
Factor Level Combinations

GLM 3


BL E1 E2 E3 M1 M2 M3 M4
control
crowding
crowding.hyp
Tail beat frequency
4.0
3.5
3.0
2.5
2.0
BL E1 E2 E3 M1 M2 M3 M4
BL E1 E2 E3 M1 M2 M3 M4
Phase

APPENDIX 3

STATISTICAL OUTPUT: Tail beat frequency

Analysis of Variance Table.

```
Response: TB.frequency
                  Df  Sum Sq Mean Sq F value    Pr(>F)
Treatment          2  41.121 20.5606 51.3802 < 2.2e-16 ***
Phase              7  50.418  7.2026 17.9991 < 2.2e-16 ***
Treatment:Phase   13  38.367  2.9513  7.3752 1.088e-12 ***
Residuals        322 128.853  0.4002
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Tukey HSD test. Significant results are highlighted yellow.

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = TB.frequency~Treatment*Phase)

$Treatment
                           diff       lwr       upr     p adj
crowding-control      0.5275000 0.3352077 0.7197923 0.0000000
crowding.hyp-control  0.8420238 0.6429823 1.0410653 0.0000000
crowding.hyp-crowding 0.3145238 0.1154823 0.5135653 0.0006842

$Phase
              diff         lwr        upr     p adj
T1-P   0.76000000  0.35311845  1.1668815 0.0000008
T2-P   0.62444444  0.21756290  1.0313260 0.0001127
T3-P   0.50386905  0.04896165  0.9587764 0.0183291
M1-P  -0.06888889 -0.47577044  0.3379927 0.9995738
M2-P  -0.27333333 -0.68021488  0.1335482 0.4502081
M3-P  -0.06888889 -0.47577044  0.3379927 0.9995738
M4-P  -0.22222222 -0.62910377  0.1846593 0.7090046
T2-T1 -0.13555556 -0.54243710  0.2713260 0.9717595
T3-T1 -0.25613095 -0.71103835  0.1987764 0.6756444
M1-T1 -0.82888889 -1.23577044 -0.4220073 0.0000000
M2-T1 -1.03333333 -1.44021488 -0.6264518 0.0000000
M3-T1 -0.82888889 -1.23577044 -0.4220073 0.0000000
M4-T1 -0.98222222 -1.38910377 -0.5753407 0.0000000
T3-T2 -0.12057540 -0.57548280  0.3343320 0.9925692
M1-T2 -0.69333333 -1.10021488 -0.2864518 0.0000098
M2-T2 -0.89777778 -1.30465933 -0.4908962 0.0000000
M3-T2 -0.69333333 -1.10021488 -0.2864518 0.0000098
M4-T2 -0.84666667 -1.25354822 -0.4397851 0.0000000
M1-T3 -0.57275794 -1.02766534 -0.1178505 0.0036469
M2-T3 -0.77720238 -1.23210978 -0.3222950 0.0000092
M3-T3 -0.57275794 -1.02766534 -0.1178505 0.0036469
M4-T3 -0.72609127 -1.18099867 -0.2711839 0.0000477
M2-M1 -0.20444444 -0.61132599  0.2024371 0.7890557
M3-M1  0.00000000 -0.40688155  0.4068815 1.0000000
M4-M1 -0.15333333 -0.56021488  0.2535482 0.9450674
M3-M2  0.20444444 -0.20243710  0.6113260 0.7890557
M4-M2  0.05111111 -0.35577044  0.4579927 0.9999424
M4-M3 -0.15333333 -0.56021488  0.2535482 0.9450674
```

```
$Treatment:Phase
                                          diff           lwr          upr     p adj
crowding:P-control:P                -6.666667e-03 -0.8542560090  0.840922676 1.0000000
crowding.hyp:P-control:P             6.133333e-01 -0.2342560090  1.460922676 0.5540190
control:T1-control:P                 2.000000e-02 -0.8275893423  0.867589342 1.0000000
control:T2-control:P                -1.200000e-01 -0.9675893423  0.727589342 1.0000000
control:T3-control:P                -1.333333e-01 -0.9809226756  0.714256009 1.0000000
control:M1-control:P                -1.133333e-01 -0.9609226756  0.734256009 1.0000000
control:M2-control:P                -6.000000e-01 -1.4475893423  0.247589342 0.5996353
control:M3-control:P                -2.000000e-01 -1.0475893423  0.647589342 0.9999999
control:M4-control:P                 2.466667e-01 -0.6009226756  1.094256009 0.9999946
crowding.hyp:P-crowding:P            6.200000e-01 -0.2275893423  1.467589342 0.5311941
crowding:T1-crowding:P               1.466667e+00  0.6190773244  2.314256009 0.0000002
crowding:T2-crowding:P               1.200000e+00  0.3524106577  2.047589342 0.0000947
crowding:T3-crowding:P               1.166667e+00  0.3190773244  2.014256009 0.0001896
crowding:M1-crowding:P               3.266667e-01 -0.5209226756  1.174256009 0.9993554
crowding:M2-crowding:P              -5.333333e-02 -0.9009226756  0.794256009 1.0000000
crowding:M3-crowding:P              -8.000000e-02 -0.9275893423  0.767589342 1.0000000
crowding:M4-crowding:P              -6.533333e-01 -1.5009226756  0.194256009 0.4198008
crowding.hyp:T1-crowding.hyp:P       7.933333e-01 -0.0542560090  1.640922676 0.1019614
crowding.hyp:T2-crowding.hyp:P       7.933333e-01 -0.0542560090  1.640922676 0.1019614
crowding.hyp:M1-crowding.hyp:P      -4.200000e-01 -1.2675893423  0.427589342 0.9801938
crowding.hyp:M2-crowding.hyp:P      -1.666667e-01 -1.0142560090  0.680922676 1.0000000
crowding.hyp:M3-crowding.hyp:P       7.333333e-02 -0.7742560090  0.920922676 1.0000000
crowding.hyp:M4-crowding.hyp:P      -2.600000e-01 -1.1075893423  0.587589342 0.9999859
crowding:T1-control:T1               1.440000e+00  0.5924106577  2.287589342 0.0000004
crowding.hyp:T1-control:T1           1.386667e+00  0.5390773244  2.234256009 0.0000014
control:T2-control:T1               -1.400000e-01 -0.9875893423  0.707589342 1.0000000
control:T3-control:T1               -1.533333e-01 -1.0009226756  0.694256009 1.0000000
control:M1-control:T1               -1.333333e-01 -0.9809226756  0.714256009 1.0000000
control:M2-control:T1               -6.200000e-01 -1.4675893423  0.227589342 0.5311941
control:M3-control:T1               -2.200000e-01 -1.0675893423  0.627589342 0.9999994
control:M4-control:T1                2.266667e-01 -0.6209226756  1.074256009 0.9999989
crowding.hyp:T1-crowding:T1         -5.333333e-02 -0.9009226756  0.794256009 1.0000000
crowding:T2-crowding:T1             -2.666667e-01 -1.1142560090  0.580922676 0.9999778
crowding:T3-crowding:T1             -3.000000e-01 -1.1475893423  0.547589342 0.9998341
crowding:M1-crowding:T1             -1.140000e+00 -1.9875893423 -0.292410658 0.0003259
crowding:M2-crowding:T1             -1.520000e+00 -2.3675893423 -0.672410658 0.0000001
crowding:M3-crowding:T1             -1.546667e+00 -2.3942560090 -0.699077324 0.0000000
crowding:M4-crowding:T1             -2.120000e+00 -2.9675893423 -1.272410658 0.0000000
crowding.hyp:T2-crowding.hyp:T1 -8.881784e-16 -0.8475893423  0.847589342 1.0000000
crowding.hyp:M1-crowding.hyp:T1 -1.213333e+00 -2.0609226756 -0.365743991 0.0000714
crowding.hyp:M2-crowding.hyp:T1 -9.600000e-01 -1.8075893423 -0.112410658 0.0089696
crowding.hyp:M3-crowding.hyp:T1 -7.200000e-01 -1.5675893423  0.127589342 0.2322587
crowding.hyp:M4-crowding.hyp:T1 -1.053333e+00 -1.9009226756 -0.205743991 0.0017386
crowding:T2-control:T2               1.313333e+00  0.4657439910  2.160922676 0.0000078
crowding.hyp:T2-control:T2           1.526667e+00  0.6790773244  2.374256009 0.0000000
control:T3-control:T2               -1.333333e-02 -0.8609226756  0.834256009 1.0000000
control:M1-control:T2                6.666667e-03 -0.8409226756  0.854256009 1.0000000
control:M2-control:T2               -4.800000e-01 -1.3275893423  0.367589342 0.9195638
control:M3-control:T2               -8.000000e-02 -0.9275893423  0.767589342 1.0000000
control:M4-control:T2                3.666667e-01 -0.4809226756  1.214256009 0.9965031
crowding.hyp:T2-crowding:T2          2.133333e-01 -0.6342560090  1.060922676 0.9999997
crowding:T3-crowding:T2             -3.333333e-02 -0.8809226756  0.814256009 1.0000000
crowding:M1-crowding:T2             -8.733333e-01 -1.7209226756 -0.025743991 0.0346727
crowding:M2-crowding:T2             -1.253333e+00 -2.1009226756 -0.405743991 0.0000301
crowding:M3-crowding:T2             -1.280000e+00 -2.1275893423 -0.432410658 0.0000167
crowding:M4-crowding:T2             -1.853333e+00 -2.7009226756 -1.005743991 0.0000000
crowding.hyp:M1-crowding.hyp:T2 -1.213333e+00 -2.0609226756 -0.365743991 0.0000714
crowding.hyp:M2-crowding.hyp:T2 -9.600000e-01 -1.8075893423 -0.112410658 0.0089696
```

crowding.hyp:M3-crowding.hyp:T2	-7.200000e-01	-1.5675893423	0.127589342	0.2322587
crowding.hyp:M4-crowding.hyp:T2	-1.053333e+00	-1.9009226756	-0.205743991	0.0017386
crowding:T3-control:T3	1.293333e+00	0.4457439910	2.140922676	0.0000123
control:M1-control:T3	2.000000e-02	-0.8275893423	0.867589342	1.0000000
control:M2-control:T3	-4.666667e-01	-1.3142560090	0.380922676	0.9385357
control:M3-control:T3	-6.666667e-02	-0.9142560090	0.780922676	1.0000000
control:M4-control:T3	3.800000e-01	-0.4675893423	1.227589342	0.9943250
crowding:M1-crowding:T3	-8.400000e-01	-1.6875893423	0.007589342	0.0555123
crowding:M2-crowding:T3	-1.220000e+00	-2.0675893423	-0.372410658	0.0000619
control:M3-crowding:T3	-1.360000e+00	-2.2075893423	-0.512410658	0.0000027
crowding:M3-crowding:T3	-1.246667e+00	-2.0942560090	-0.399077324	0.0000348
crowding:M4-crowding:T3	-1.820000e+00	-2.6675893423	-0.972410658	0.0000000
crowding:M1-control:M1	4.333333e-01	-0.4142560090	1.280922676	0.9717174
crowding.hyp:M1-control:M1	3.066667e-01	-0.5409226756	1.154256009	0.9997626
control:M2-control:M1	-4.866667e-01	-1.3342560090	0.360922676	0.9087290
control:M3-control:M1	-8.666667e-02	-0.9342560090	0.760922676	1.0000000
control:M4-control:M1	3.600000e-01	-0.4875893423	1.207589342	0.9972933
crowding.hyp:M1-crowding:M1	-1.266667e-01	-0.9742560090	0.720922676	1.0000000
crowding:M2-crowding:M1	-3.800000e-01	-1.2275893423	0.467589342	0.9943250
crowding:M3-crowding:M1	-4.066667e-01	-1.2542560090	0.440922676	0.9865242
crowding:M4-crowding:M1	-9.800000e-01	-1.8275893423	-0.132410658	0.0064076
crowding.hyp:M2-crowding.hyp:M1	2.533333e-01	-0.5942560090	1.100922676	0.9999911
crowding.hyp:M3-crowding.hyp:M1	4.933333e-01	-0.3542560090	1.340922676	0.8969709
crowding.hyp:M4-crowding.hyp:M1	1.600000e-01	-0.6875893423	1.007589342	1.0000000
crowding:M2-control:M2	5.400000e-01	-0.3075893423	1.387589342	0.7890703
crowding.hyp:M2-control:M2	1.046667e+00	0.1990773244	1.894256009	0.0019662
control:M3-control:M2	4.000000e-01	-0.4475893423	1.247589342	0.9890107
control:M4-control:M2	8.466667e-01	-0.0009226756	1.694256009	0.0506440
crowding.hyp:M2-crowding:M2	5.066667e-01	-0.3409226756	1.354256009	0.8706569
crowding:M3-crowding:M2	-2.666667e-02	-0.8742560090	0.820922676	1.0000000
crowding:M4-crowding:M2	-6.000000e-01	-1.4475893423	0.247589342	0.5996353
crowding.hyp:M3-crowding.hyp:M2	2.400000e-01	-0.6075893423	1.087589342	0.9999967
crowding.hyp:M4-crowding.hyp:M2	-9.333333e-02	-0.9409226756	0.754256009	1.0000000
crowding:M3-control:M3	1.133333e-01	-0.7342560090	0.960922676	1.0000000
crowding.hyp:M3-control:M3	8.866667e-01	0.0390773244	1.734256009	0.0284936
control:M4-control:M3	4.466667e-01	-0.4009226756	1.294256009	0.9606894
crowding.hyp:M3-crowding:M3	7.733333e-01	-0.0742560090	1.620922676	0.1297753
crowding:M4-crowding:M3	-5.733333e-01	-1.4209226756	0.274256009	0.6885449
crowding.hyp:M4-crowding.hyp:M3	-3.333333e-01	-1.1809226756	0.514256009	0.9991222
crowding:M4-control:M4	-9.066667e-01	-1.7542560090	-0.059077324	0.0210524
crowding.hyp:M4-control:M4	1.066667e-01	-0.7409226756	0.954256009	1.0000000
crowding.hyp:M4-crowding:M4	1.013333e+00	0.1657439910	1.860922676	0.0035897

APPENDIX 4

STATISTCAL OUTPUT: Tail beat amplitude

Analysis of Variance Table.

```
Response: TB.amplitude
                  Df   Sum Sq   Mean Sq F value    Pr(>F)
Treatment          2 0.031476 0.0157379 22.0767 1.035e-09 ***
Phase              7 0.015129 0.0021612  3.0317  0.004223 **
Treatment:Phase   13 0.012481 0.0009601  1.3468  0.184324
Residuals        322 0.229545 0.0007129
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Tukey HSD test. Significant results are highlighted green.

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = TB.amplitude~Treatment*Phase)

$Treatment
                             diff          lwr          upr     p adj
crowding-control       0.02190833  0.013792214  0.030024452 0.0000000
crowding.hyp-control   0.00490000 -0.003500981  0.013300981 0.3561137
crowding.hyp-crowding -0.01700833 -0.025409315 -0.008607352 0.0000085

$Phase
               diff          lwr          upr     p adj
T1-P  -0.0021777778 -0.019351103  0.014995548 0.9999386
T2-P   0.0026000000 -0.014573325  0.019773325 0.9997974
T3-P   0.0022819444 -0.016918417  0.021482306 0.9999605
M1-P   0.0077777778 -0.009395548  0.024951103 0.8650866
M2-P  -0.0067777778 -0.023951103  0.010395548 0.9303527
M3-P  -0.0114888889 -0.028662214  0.005684437 0.4558529
M4-P  -0.0116222222 -0.028795548  0.005551103 0.4401422
T2-T1  0.0047777778 -0.012395548  0.021951103 0.9900502
T3-T1  0.0044597222 -0.014740639  0.023660084 0.9967134
M1-T1  0.0099555556 -0.007217770  0.027128881 0.6417615
M2-T1 -0.0046000000 -0.021773325  0.012573325 0.9920801
M3-T1 -0.0093111111 -0.026484437  0.007862214 0.7166919
M4-T1 -0.0094444444 -0.026617770  0.007728881 0.7016137
T3-T2 -0.0003180556 -0.019518417  0.018882306 1.0000000
M1-T2  0.0051777778 -0.011995548  0.022351103 0.9840106
M2-T2 -0.0093777778 -0.026551103  0.007795548 0.7091853
M3-T2 -0.0140888889 -0.031262214  0.003084437 0.1978652
M4-T2 -0.0142222222 -0.031395548  0.002951103 0.1880017
M1-T3  0.0054958333 -0.013704528  0.024696195 0.9882143
M2-T3 -0.0090597222 -0.028260084  0.010140639 0.8381232
M3-T3 -0.0137708333 -0.032971195  0.005429528 0.3616293
M4-T3 -0.0139041667 -0.033104528  0.005296195 0.3488334
M2-M1 -0.0145555556 -0.031728881  0.002617770 0.1648755
M3-M1 -0.0192666667 -0.036439992 -0.002093341 0.0158980
M4-M1 -0.0194000000 -0.036573325 -0.002226675 0.0147002
M3-M2 -0.0047111111 -0.021884437  0.012462214 0.9908540
```

```
M4-M2 -0.0048444444 -0.022017770  0.012328881 0.9891924
M4-M3 -0.0001333333 -0.017306659  0.017039992 1.0000000

$`Treatment:Phase`
                                          diff          lwr           upr     p adj
crowding:P-control:P                 0.0100000000 -0.025774362  0.0457743616 0.9999974
crowding.hyp:P-control:P            -0.0172000000 -0.052974362  0.0185743616 0.9861781
control:T1-control:P                -0.0176666667 -0.053441028  0.0181076949 0.9809682
control:T2-control:P                -0.0178666667 -0.053641028  0.0179076949 0.9783065
control:T3-control:P                -0.0080000000 -0.043774362  0.0277743616 1.0000000
control:M1-control:P                -0.0033333333 -0.039107695  0.0324410283 1.0000000
control:M2-control:P                -0.0260000000 -0.061774362  0.0097743616 0.5448528
control:M3-control:P                -0.0169333333 -0.052707695  0.0188410283 0.9885947
control:M4-control:P                -0.0219333333 -0.057707695  0.0138410283 0.8414593
crowding.hyp:P-crowding:P           -0.0272000000 -0.062974362  0.0085743616 0.4488204
crowding:T1-crowding:P              -0.0008000000 -0.036574362  0.0349743616 1.0000000
crowding:T2-crowding:P               0.0099333333 -0.025841028  0.0457076949 0.9999977
crowding:T3-crowding:P               0.0018000000 -0.033974362  0.0375743616 1.0000000
crowding:M1-crowding:P               0.0138000000 -0.021974362  0.0495743616 0.9993465
crowding:M2-crowding:P              -0.0058000000 -0.041574362  0.0299743616 1.0000000
crowding:M3-crowding:P              -0.0236000000 -0.059374362  0.0121743616 0.7332138
crowding:M4-crowding:P              -0.0118000000 -0.047574362  0.0239743616 0.9999494
crowding.hyp:T1-crowding.hyp:P       0.0119333333 -0.023841028  0.0477076949 0.9999386
crowding.hyp:T2-crowding.hyp:P       0.0157333333 -0.020041028  0.0515076949 0.9956165
crowding.hyp:M1-crowding.hyp:P       0.0128666667 -0.022907695  0.0486410283 0.9997844
crowding.hyp:M2-crowding.hyp:P       0.0114666667 -0.024307695  0.0472410283 0.9999692
crowding.hyp:M3-crowding.hyp:P       0.0060666667 -0.029707695  0.0418410283 1.0000000
crowding.hyp:M4-crowding.hyp:P      -0.0011333333 -0.036907695  0.0346410283 1.0000000
crowding:T1-control:T1               0.0268666667 -0.008907695  0.0626410283 0.4750964
crowding.hyp:T1-control:T1           0.0124000000 -0.023374362  0.0481743616 0.9998827
control:T2-control:T1               -0.0002000000 -0.035974362  0.0355743616 1.0000000
control:T3-control:T1                0.0096666667 -0.026107695  0.0454410283 0.9999986
control:M1-control:T1                0.0143333333 -0.021441028  0.0501076949 0.9988388
control:M2-control:T1               -0.0083333333 -0.044107695  0.0274410283 0.9999999
control:M3-control:T1                0.0007333333 -0.035041028  0.0365076949 1.0000000
control:M4-control:T1               -0.0042666667 -0.040041028  0.0315076949 1.0000000
crowding.hyp:T1-crowding:T1         -0.0144666667 -0.050241028  0.0213076949 0.9986678
crowding:T2-crowding:T1              0.0107333333 -0.025041028  0.0465076949 0.9999905
crowding:T3-crowding:T1              0.0026000000 -0.033174362  0.0383743616 1.0000000
crowding:M1-crowding:T1              0.0146000000 -0.021174362  0.0503743616 0.9984752
crowding:M2-crowding:T1             -0.0050000000 -0.040774362  0.0307743616 1.0000000
crowding:M3-crowding:T1             -0.0228000000 -0.058574362  0.0129743616 0.7885386
crowding:M4-crowding:T1             -0.0110000000 -0.046774362  0.0247743616 0.9999852
crowding.hyp:T2-crowding.hyp:T1      0.0038000000 -0.031974362  0.0395743616 1.0000000
crowding.hyp:M1-crowding.hyp:T1      0.0009333333 -0.034841028  0.0367076949 1.0000000
crowding.hyp:M2-crowding.hyp:T1     -0.0004666667 -0.036241028  0.0353076949 1.0000000
crowding.hyp:M3-crowding.hyp:T1     -0.0058666667 -0.041641028  0.0299076949 1.0000000
crowding.hyp:M4-crowding.hyp:T1     -0.0130666667 -0.048841028  0.0227076949 0.9997234
crowding:T2-control:T2               0.0378000000  0.002025638  0.0735743616 0.0249274
crowding.hyp:T2-control:T2           0.0164000000 -0.019374362  0.0521743616 0.9924000
control:T3-control:T2                0.0098666667 -0.025907695  0.0456410283 0.9999980
control:M1-control:T2                0.0145333333 -0.021241028  0.0503076949 0.9985743
control:M2-control:T2               -0.0081333333 -0.043907695  0.0276410283 1.0000000
control:M3-control:T2                0.0009333333 -0.034841028  0.0367076949 1.0000000
control:M4-control:T2               -0.0040666667 -0.039841028  0.0317076949 1.0000000
crowding.hyp:T2-crowding:T2         -0.0214000000 -0.057174362  0.0143743616 0.8699033
crowding:T3-crowding:T2             -0.0081333333 -0.043907695  0.0276410283 1.0000000
crowding:M1-crowding:T2              0.0038666667 -0.031907695  0.0396410283 1.0000000
crowding:M2-crowding:T2             -0.0157333333 -0.051507695  0.0200410283 0.9956165
crowding:M3-crowding:T2             -0.0335333333 -0.069307695  0.0022410283 0.1005079
crowding:M4-crowding:T2             -0.0217333333 -0.057507695  0.0140410283 0.8525087
crowding.hyp:M1-crowding.hyp:T2     -0.0028666667 -0.038641028  0.0329076949 1.0000000
```

crowding.hyp:M2-crowding.hyp:T2	-0.0042666667	-0.040041028	0.0315076949	1.0000000
crowding.hyp:M3-crowding.hyp:T2	-0.0096666667	-0.045441028	0.0261076949	0.9999986
crowding.hyp:M4-crowding.hyp:T2	-0.0168666667	-0.052641028	0.0189076949	0.9891417
crowding:T3-control:T3	0.0198000000	-0.015974362	0.0555743616	0.9353159
control:M1-control:T3	0.0046666667	-0.031107695	0.0404410283	1.0000000
control:M2-control:T3	-0.0180000000	-0.053774362	0.0177743616	0.9763754
control:M3-control:T3	-0.0089333333	-0.044707695	0.0268410283	0.9999997
control:M4-control:T3	-0.0139333333	-0.049707695	0.0218410283	0.9992426
crowding:M1-crowding:T3	0.0120000000	-0.023774362	0.0477743616	0.9999325
crowding:M2-crowding:T3	-0.0076000000	-0.043374362	0.0281743616	1.0000000
crowding:M3-crowding:T3	-0.0254000000	-0.061174362	0.0103743616	0.5935192
crowding:M4-crowding:T3	-0.0136000000	-0.049374362	0.0221743616	0.9994788
crowding:M1-control:M1	0.0271333333	-0.008641028	0.0629076949	0.4540410
crowding.hyp:M1-control:M1	-0.0010000000	-0.036774362	0.0347743616	1.0000000
control:M2-control:M1	-0.0226666667	-0.058441028	0.0131076949	0.7971883
control:M3-control:M1	-0.0136000000	-0.049374362	0.0221743616	0.9994788
control:M4-control:M1	-0.0186000000	-0.054374362	0.0171743616	0.9659793
crowding.hyp:M1-crowding:M1	-0.0281333333	-0.063907695	0.0076410283	0.3780537
crowding:M2-crowding:M1	-0.0196000000	-0.055374362	0.0161743616	0.9414445
crowding:M3-crowding:M1	-0.0374000000	-0.073174362	-0.0016256384	0.0287327
crowding:M4-crowding:M1	-0.0256000000	-0.061374362	0.0101743616	0.5773202
crowding.hyp:M2-crowding.hyp:M1	-0.0014000000	-0.037174362	0.0343743616	1.0000000
crowding.hyp:M3-crowding.hyp:M1	-0.0068000000	-0.042574362	0.0289743616	1.0000000
crowding.hyp:M4-crowding.hyp:M1	-0.0140000000	-0.049774362	0.0217743616	0.9991854
crowding:M2-control:M2	0.0302000000	-0.005574362	0.0659743616	0.2428683
crowding.hyp:M2-control:M2	0.0202666667	-0.015507695	0.0560410283	0.9192973
control:M3-control:M2	0.0090666667	-0.026707695	0.0448410283	0.9999996
control:M4-control:M2	0.0040666667	-0.031707695	0.0398410283	1.0000000
crowding.hyp:M2-crowding:M2	-0.0099333333	-0.045707695	0.0258410283	0.9999977
crowding:M3-crowding:M2	-0.0178000000	-0.053574362	0.0179743616	0.9792243
crowding:M4-crowding:M2	-0.0060000000	-0.041774362	0.0297743616	1.0000000
crowding.hyp:M3-crowding.hyp:M2	-0.0054000000	-0.041174362	0.0303743616	1.0000000
crowding.hyp:M4-crowding.hyp:M2	-0.0126000000	-0.048374362	0.0231743616	0.9998470
crowding:M3-control:M3	0.0033333333	-0.032441028	0.0391076949	1.0000000
crowding.hyp:M3-control:M3	0.0058000000	-0.029974362	0.0415743616	1.0000000
control:M4-control:M3	-0.0050000000	-0.040774362	0.0307743616	1.0000000
crowding.hyp:M3-crowding:M3	0.0024666667	-0.033307695	0.0382410283	1.0000000
crowding:M4-crowding:M3	0.0118000000	-0.023974362	0.0475743616	0.9999494
crowding.hyp:M4-crowding.hyp:M3	-0.0072000000	-0.042974362	0.0285743616	1.0000000
crowding:M4-control:M4	0.0201333333	-0.015641028	0.0559076949	0.9241243
crowding.hyp:M4-control:M4	0.0036000000	-0.032174362	0.0393743616	1.0000000
crowding.hyp:M4-crowding:M4	-0.0165333333	-0.052307695	0.0192410283	0.9915649