Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Tine Elisabeth Dahl Brusevold

Behavioural traits correlate in zebrafish (*Danio rerio*) selected for high and low upper thermal tolerance

Master's thesis in Natural Science with Teacher Education
Supervisor: Jonathan Wright
Co-supervisors: Mette Helene Finnøen, Fredrik Jutfelt, Rachael Morgan
June 2019

NTNU
Norwegian University of
Science and Technology

Jutfelt Fish
Ecophysiology lab

Tine Elisabeth Dahl Brusevold

Behavioural traits correlate in zebrafish (*Danio rerio*) selected for high and low upper thermal tolerance

Master’s thesis in Natural Science with Teacher Education
Supervisor: Jonathan Wright
Co-supervisors: Mette Helene Finnøen, Fredrik Jutfelt, Rachael Morgan
June 2019

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of
Science and Technology

Acknowledgements

Firstly, and most profoundly, I would like to thank my co-supervisor Mette Helene Finnøen for priceless patience, council, help and guidance throughout this project, for teaching me R, statistics and EthoVision and for being a friend. You've been exceptional. A huge thanks is also given to my supervisor Jonathan Wright, for your guidance, and for keeping me on the right track this year. I would also like to thank my other co-supervisors, Rachael Morgan for all your help and for always being positive and cheerful and Fredrik Jutfelt, for all your help, for creating such an awesome working environment for the group and of course – for the beautiful zebrafish. Thanks to Hanne Margrethe Hildrum, for being a wonderful collaborator. You have taught me so much. To Anna and Eirik, for all the help and inspiration you've provided me. Thanks to Miriam, who is always available and on top of everything going on in the lab. Being part of this group has been amazing, and probably the most academically educational experience of my life. Last but not least, I would like to thank my family; Hege and our children, for your patience and support. You are my biggest motivation for managing to complete this. I love you. This is for you.

Abstract

Freshwater systems are particularly vulnerable to climate change, so research on freshwater organisms in light of temperature change is important. In this study, zebrafish that have been artificially selected through four generations for their upper thermal tolerance, were tested in behavioural assays at two different temperatures, 26˚C and 30˚C. I hypothesised that if significant differences were found between these lines, that it would be because of one of two reasons: Either a) the behavioural traits were consistently suitable along a shy – bold continuum, and could therefore be explained by the pace-of-life syndrome (POLS). Or b) that the behaviours did not suit this continuum, but rather showed that selection on high upper thermal tolerance is in fact selection on high quality individuals. Behaviours did covary within each line, and the individuals from the line selected for low upper tolerance showed more plasticity in their behaviour. I found that there were significant effects for the fixed effect interaction term temperature*line in many of the investigated behaviours, including activity, distance to surface, distance to a novel object and latency to enter the surface. This study showed more evidence towards the second hypothesis, as the behaviours did covary – but not along a shy - bold continuum as would have been expected if there was presence of a behavioural syndrome fitting with POLS. In addition, fish selected for low tolerance of CTmax were less consistent in their behaviour in the two assay temperatures. The results in this study suggest that selection on high thermal tolerance is in fact selection on high quality individuals.

Sammendrag

Ferskvannssystemer er spesielt sårbare for klimaforandringer, så forskning på ferskvannsorganismer i lys av temperaturforandringer er viktig. I denne studien, ble sebrafisk selektert gjennom fire generasjoner for øvre termisk toleranse, testet i atferdsforsøk i to ulike temperaturer, 26˚C og 30˚C. Jeg hypotisterte at hvis signifikante ulikheter ble funnet mellom de ulike linjene, ville det være på bakgrunn av en av to grunner: Enten a) atferdstrekkene var konsekvent plassert på spekter mellom de mest sjenerte og forsikte individene og de uredde, aggressive individene, som videre kan forklares med et «pace-of-life»-syndrom (POLS). Eller b) at atferdene ikke passer sammen inn i dette spektrumet, men heller viste at seleksjon på høy øvre termisk toleranse, faktisk er seleksjon på høy-kvalitets individer. Kovarians mellom atferder ble påvist innad i linjene, og individer fra Lav toleranse linjen viste mer plastisitet i sine atferder. Jeg fant at det var signifikante effekter for interaksjonen temperatur*linje i flere av atferdene, deriblant aktivitet, distanse til overflaten, distanse til et ukjent objekt og latens til å entre overflaten. Denne studien viser mer støtte til hypotese b, ettersom atferdene kovarierte, men ikke på en måte som er kompatibelt med et POLS. I tillegg var fisk selektert for lav øvre termisk toleranse mindre konsise i sine atferder i de to temperaturene. Disse resultatene peker mot seleksjon på høy øvre termisk toleranse egentlig er seleksjon på høy-kvalitets individer.

Table of contents

Introduction

Vulnerability to climate change is greater for freshwater systems than for many other ecological systems for three reasons in particular: (i) most freshwater species are site bound and physically unable to migrate; (ii) the temperature of the water and availability of the water itself is highly dependent on climate; and (iii) many freshwater systems are already suffering stress from exploitation and other human activities (Woodward et al., 2010). In addition, extreme heatwaves and temperature fluctuations are expected to increase in frequency as well as intensity following predicted climate change (Seneviratne et al., 2014). As a result, Woodward et al. (2010) claim that climate change is the biggest rising threat to global biodiversity and local ecosystems including freshwater ones.

Temperature is considered the “abiotic master factor” for trait determination in animals (López-Olmeda & Sánchez-Vázquez, 2011). The effect of temperature on freshwater fish, such as zebrafish (*Danio rerio*) the study species here, can be studied by determining the critical thermal maximum (CTmax). CTmax is the upper temperature at which an individual can function before its locomotor ability is lost, seen as disorganised swimming and loss of equilibrium (Morgan et al., 2018). This measure can tell us something about the impact of different temperature changes, such as extreme temperature differences and fluctuations in the face of climate change (Zhang & Kieffer, 2014). Temperature also affects many other aspects of physiology and morphology, such as growth, reproduction and locomotion (Schulte et al., 2011), and the distribution of species and migration as a result of thermal preference (Rey et al., 2015). Temperature also affects behavioural traits such as activity, speed, foraging and shoaling (Bennett, 1980; Biro et al., 2009; Brodie & Russell, 1999; López-Olmeda & Sánchez-Vázquez, 2011). For ectotherms, elevated temperatures mean higher metabolism on a population level (Brown et al., 2004). Metabolic rate can determine a large variety of attributes, from food requirements to developmental and mortality rates – both at the individual and population level (Brown et al., 2004). For example, arctic fish generally have lower metabolism and need to eat and move less than fish from warmer regions (López-Olmeda & Sánchez-Vázquez, 2011).

Trait correlations in animals are common and selection on one trait will often affect the evolution of other traits, both within and across behavioural, physiological and morphological traits (Kern et al., 2016). The proximate mechanisms behind this can be explained by genetic pleiotropy or linkage disequilibrium, and/or integrated plasticity during development (developmental plasticity) or by other less long-lasting environmental effects such as habituation or acclimation (Sih et al., 2004). However, both ultimate and proximate reasons for trait correlations can be explained by correlational selection.

Animals with lower metabolic rates are not as dependent on high food intakes and therefore do not need to compete or search for it as extensively. Size, speed and curiosity may thus be traits that are linked with physiological traits determining nutritional need. Careau et al. (2008) point out that the behavioural responses across individuals are a result of their genes and functions of the neuroendocrine system. This shows that physiological mechanisms may be a direct source to some of the consistent behavioural differences we see both within and between populations and species.

In previous artificial selection studies on behavioural traits (Wisenden et al., 2011; Wong et al., 2012), the artificial selection tends to be on the behaviour itself, whereas in this study we are investigating if selection on a physiological trait has meant selection on behavioural traits as well. Roy & Bhat (2018) studied the effects of physiological traits (i.e. sex and body size) correlating with behavioural traits, and found that both predation pressure and the physiological parameters played a role in the covariances between pairs of traits.

The study of behavioural syndromes can play an important role in the connection between areas like genetics, neuroendocrine mechanisms, evolution and ecology (Sih et al., 2004). This is the main phenomena that I will focus on in this thesis in its links to thermal plasticity and the evolution of CTmax. Many analogous terms exist in the literature to describe the same or similar attributes, such as personality, behavioural syndrome and temperament (Kern et al., 2016), and coping styles (Coppens et al., 2010). To avoid confusion, I will mainly use the terminology of Carter et. al (2013) and Dingemanse (2010a). To clarify, the terms related to behaviour used in this thesis are defined below in Table 1.

Fish that are considered shy should to be more plastically adjusted to the environment that they are in. This may be explained by a need for security, as shy individuals tend to be more philopatric, and bold individuals disperse more (Dingemanse et al., 2003). A fish that is adjusting to the environment and is able to keep expressing different levels of the same behaviour can thus show consistently high or low levels of behaviour whilst also being plastic (Dingemanse, et al., 2010a). I will investigate behavioural plasticity in response to temperature in various behavioural traits.

TABLE 1, DEFINITIONS OF TERMS RELATED TO THIS BEHAVIOURAL STUDY

Term	**Definition**
Personality	A consistently expressed behavioural trait, for a single behaviour, that is statistically repeatable over time among individuals in a population.
Behavioural syndrome	A collection of behavioural traits showing personality (see above) within a population that repeatably covary, either positively or negatively.
Bold	"Bold" is a relative term, describing the characteristic or trait of the behaviour in question. A fish is considered bold when it shows faster approach to a novel object, with little or no freezing behaviour and little or no bottom dwelling. Bold is considered one side of the trade-off between momentarily security (shy) and some risky but desirable gain, like food, leisure or a mate under danger of predation. When an individual rather chooses the desirable gain over the momentary security, the exhibited behaviour is characterized as bold.
Shy	"Shy" is relative to bold. A shy fish will not approach a novel object too soon (not be curious), show more freezing behaviour and more bottom dwelling. Shy behaviour will mean that the fish in question more often chooses security over gain,
Plasticity	**Behavioural plasticity** means here the ability to reversibly adjust the level of a behavioural trait to the environment, e.g. be less active in the presence of a predator. **Physiological and biochemical plasticity** means here the ability to cope physiologically with surroundings, in order to maintain the adaptive level of a behavioural trait.

The main aim of this project was to see if artificial selection on upper thermal tolerance (CTmax) has led to associated selection on certain behavioural traits. The subject fish used, were the fourth-generation zebrafish selected for high upper tolerance (called High or H), low upper tolerance (called Low or L) and a control group from the entire spectrum of tolerance (called Random or R). The individual zebrafish in this study were tested in one assay involving a novel tank (NT) followed by a novel object test (NO) in the same tank, called the NT treatment and the NO treatment, respectively. The novel object itself, will be referred to as NO. This assay was carried out in two different water temperatures for each individual in order to assess individual plasticity.

Two alternative hypotheses will be investigated in this project. The initial hypothesis is that the well-established aggression-boldness syndrome (see Garamszegi et al., 2012) of behaviours covaries with the selected lines with the prediction that high CTmax results in faster, bolder individuals, whilst low CTmax results in shy, slower individuals. As such, we might also predict that fast high CTmax individuals will be more behaviourally plastic in their responses to temperature variation than slow low CTmax individuals (see Wright et al., 2019 and Dingemanse et al., (2010a)). These behavioural syndromes may thus also be connected in a larger pace-of-life syndrome (POLS) (see Wright et al., 2019), which is the idea that life-history strategies within a population lie along a continuum with fast living, highly fecund, fast growing, highly dispersive, aggressive and bold types of individuals at one end versus slow living, low fecund, slow growing, more philopatric, non-aggressive and shy type at the other. The second and only recently formulated alternative hypothesis is that any behavioural syndrome might instead reflect differences in individual ‘quality’, because the artificial selection for high CTmax actually involved selection for high-quality individuals in many aspects. Evidence for this comes from upper constraints to the selection on high CTmax and a wider distribution of phenotypes (i.e. a wider variation of ways to be poor quality) being produced by selection for low CTmax (see Fig. B1 in Appendix B). The predictions from this second hypothesis are that high CTmax lines should show more consistent (i.e. phenotypically stable) levels of behaviour between individuals (less behavioural plasticity), and possibly higher (i.e. more adaptive) levels of each behaviour compared to low CTmax lines. If behavioural plasticity is non-adaptive, then this hypothesis would also predict smaller differences in behaviour between the two water temperatures in the high CTmax lines compared to the low CTmax lines, since these higher quality individuals are more able to make the physiological adjustments needed to produce consistent and adaptive levels of each behaviour irrespective of water temperature. The null hypothesis here can therefore be defined as no sign of a behavioural syndrome and/or no differences between selected lines in the mean behaviours or the plastic responses to the two temperatures. This project should therefore provide useful first evidence that these selected lines differ in their levels and consistency of covarying behaviours, and in behavioural plasticity in response to water temperature variation.

Methods

Study system and framework

Zebrafish are shoaling, freshwater fish, found in tropical and subtropical areas of Asia (Rey et al., 2015; Rowena et al., 2008). The zebrafish is also a common and very useful model organism because of its fast, external embryonic development (Nusslein-Volhard & Dahm, 2002). The early embryo is transparent and early development of organs can be seen. Zebrafish are therefore a popular organism for studying genetics (Reed & Jennings, 2011) and they are well suited for addressing questions related to temperature as they are eurythermal, can be acclimated for 10–36°C and they bounce back readily following acute upper thermal tolerance, critical thermal maximum (CTmax), which has been shown to be individually repeatable in zebrafish. (Morgan et al., 2018). They have a short generation time, even though being an annual species in the wild (Spence et al., 2007) and do well in captivity, making it an excellent study organism in general.

For this study, the subject fish has been selected for 4 generations on their CTmax. Highest (H or High line), lowest (L or Low) and randomly (R or Random) performing fish descending from a West Bengal wild population has been bred in the Jutfelt ecophysiology lab. The different lines have also been split in two replicates, to control for genetic confounds (Morgan et al., 2018) (see Methods for details).

Wild caught zebrafish were brought to NTNU from West Bengal, India, in 2016 by Ass. Prof. Fredrik Jut felt's ecophysiology lab. As the initial stage of the selection experiment, this first to be selected population (n=1200) was tested for their CTmax where the 33% (n=300) highest tolerant group and 33% lowest tolerant group were selected. In addition, 33% of the fish, randomly chosen from the performance results, was kept as a control line, as domestication might affect the fish. These three lines were each divided in two replicates named H1, H2, L1, L2, R1 and R2, and selected in the same manner for 4 subsequent generations (see Fig. 1). The F4 generation was used in this experiment in 2018. Each replicate was further divided into two tanks, each containing 18 fish. The tanks were named H1.1, H1.2, L1.1 etc, creating 12 tanks, randomly placed on two shelves in a temperature-controlled room (picture in Appendix C). This was to randomize any tank level effects from position in the room, such as proximity to door, airflow or different levels of daily disturbance, etc. Water temperature was kept at 28°C ±1°C by controlling room temperature. The salinity was monitored by measuring conductivity, and other levels of water quality (nitrite, pollutants, clarity) were controlled by test strips and visual daily controls. Aquaria water was changed once a week, or whenever found necessary by the daily controls (unclear water).

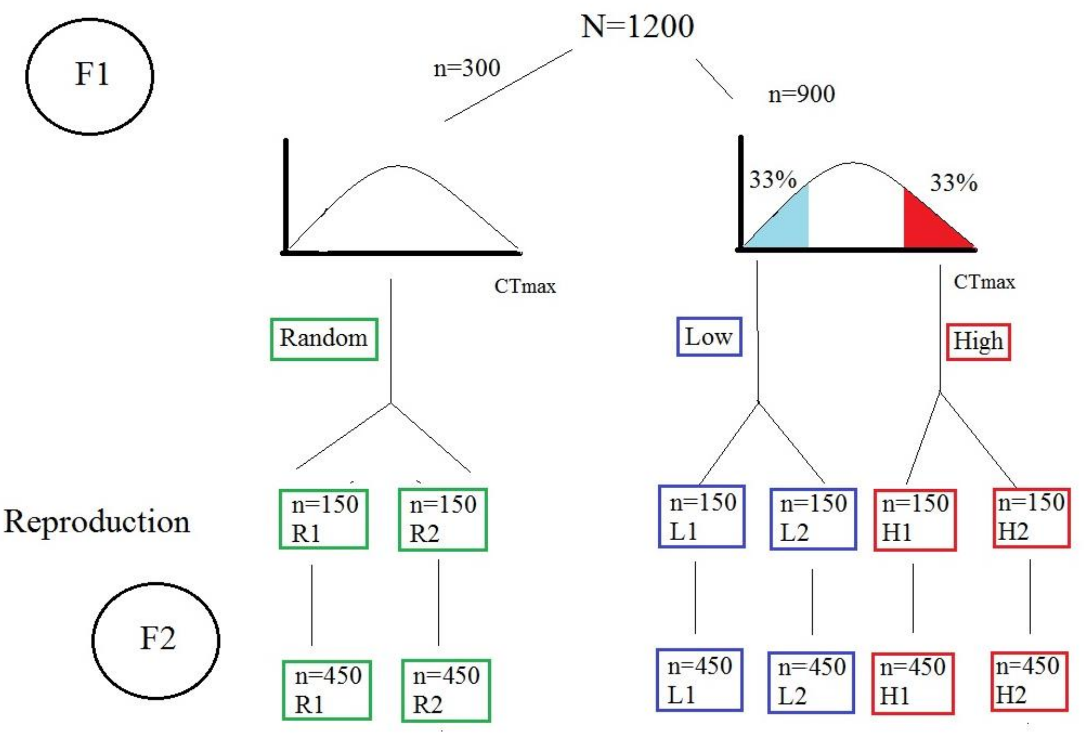


FIGURE 1, SCHEMATIC OVERVIEW OF THE FIRST TWO GENERATIONS (F1 AND F2) FOR THE THREE LINES USED IN THE EXPERIMENT. PARENT GENERATION (N=1200) WAS SUBMITTED TO CTMAX SELECTION, WHERE THE 33% HIGHEST AND LOWEST PERFORMERS WERE USED TO MAKE THE TWO MAIN LINES; HIGH (H) AND LOW (L). 33% RANDOMLY CHOSEN FISH FROM THE SELECTION WERE KEPT AS A CONTROL GROUP (R). GENERATION F4 WAS USED IN THIS EXPERIMENT (NOT VISIBLE IN FIGURE).

For identification, each fish was individually tagged (subcutaneous colouring) using visible implant elastomer tags (VIE) from Northwest Marine Technology, Inc. Shaw Island, WA, USA. Prior to tagging, the fish were anaesthetized using ~110mg/L buffered tricaine methane sulfonate (MS-222). These tags were implemented below and slightly behind the dorsal fin on both sides, using a syringe and needle. The colours used for the 18 unique combinations were red, orange, yellow, blue, green and pink (see Appendix B for details).

Body size measurements

All fish were weighed and measured twice; firstly, when they were tagged (4th and 5th of September 2018) and lastly directly after being euthanized (9th, 10th and 12th of October 2018). Weight was recorded to the nearest 0.001 gram. Length was recorded to the nearest 0.001 cm, using a digital calliper.

Specific growth rate (SGR) was then calculated using the following equation:

$$\mathrm{SGR} = \frac{\log(\mathit{length\ last}) - \log(\mathit{length\ first})}{\mathit{days\ between\ measuring}}$$

Behavioural assays

The behavioural assay tanks were designed and first used by PhD candidate Mette Helene Finnøen in 2017 (unpublished data), and measure 30x40x7cm (Fig. 2). The water column was at 25cm, low enough to ensure that no fish could jump out. The back and sides of the tanks were covered in white film to reduce visual disturbance for the fish. Eight tanks were placed on two shelves (4x2) and filmed from the side using a Kurokesu C1 IR USB camera (Vilnius, Lithuania) to record the video files.

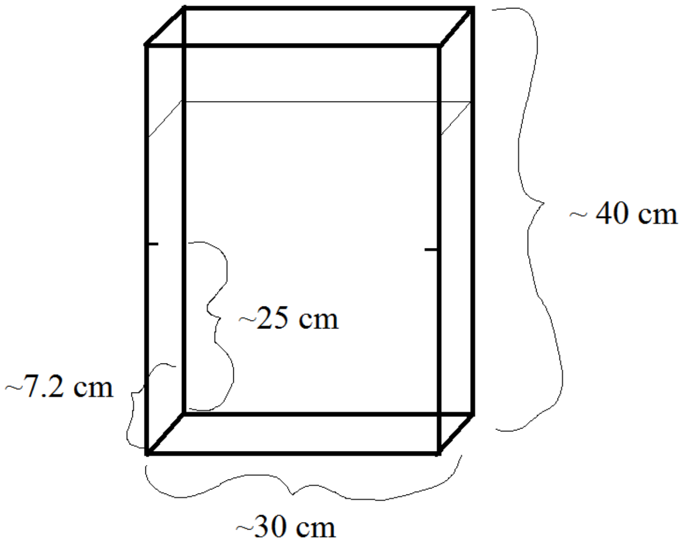


FIGURE 2, ASSAY TANK WITH MEASUREMENTS. BACK AND SIDE WALLS ARE COVERED WITH WHITE FILM, TO PREVENT FISH FROM BEING DISTURBED BY EACH OTHER AND THE SURROUNDINGS, AS MUCH AS POSSIBLE.

Each fish was exposed to the behavioural assays two times, once in 26°C and once in 30°C, in random stratified order, in order to balance orders between and within each line (Table 3). The behavioural assays were conducted with eight fish from the same tank at a time, each in individual assay tanks.

Table 2, timetable for behavioural assays. Time of day refers to the order of the assays. Each assay contained eight fish, randomly splitting each Aquaria in two assays.

Time of day	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**
	26˚C	**30˚C**	**26˚C**	**30˚C**	**Break**	**30˚C**	**26˚C**	**30˚C**	**26˚C**
1 +2	H1.1	L1.2	R2.1	H2.2		R1.1	R1.2	L1.1	L2.2
3 + 4	R1.1	H1.2	L2.1	R2.2		H1.1	L1.2	H2.1	H2.2
5 + 6		R1.2	H2.1	L2.2			H1.2	R2.1	R2.2
7 + 8			L1.1					L2.1	

Directly after all the fish had been transferred to the novel tanks, the recording was started. After 20 minutes, a novel object (Lego figure with a Lego flag, glued to a metal nut and ring, see Fig. 3) was lowered into the lower left corner of each tank, using fishing line from outside the room to minimise disturbance during the trials. The total trial time was 40mins, at which time the video was stopped. All fish were then removed and identified by their colour tags. The temperature of the water of the assay tanks was measured immediately before and after each trial. The temperature was within ±0.8˚C of the aimed temperature (26 or 30°C). Assay tanks were scrubbed between trials and the water changed. Assay water was kept in 200L barrels, added salt and Aqua Safe at a similar level to that of the home aquaria. Prior to the assays, the fish were not fed for at least 24 hours to ensure empty stomachs and as even a state of hunger as possible for all the fish.

FIGURE 3, NOVEL OBJECT FROM THE NOVEL OBJECT TREATMENT OF THE BEHAVIOURAL ASSAYS. A LEGO FIGURE WITH A LEGO FLAG GLUED TO A METAL NUT AND RING

Data collection and statistical analysis

EthoVision

The data collection was done using Noldus EthoVision XT13, which is tracking software for behavioural analysis from video recordings. It is widely used for fish and rodents and other animals (noldus.com, 2019). Using EthoVision, each tank was divided into two main zones: upper and lower; and two smaller zones: bottom and surface. Surface and bottom covered 13% each of the total water column. In addition, the space in which the NO occupied for the NO treatment was defined (Fig.4).

FIGURE 4, ARENA SETTINGS AS USED IN ETHOVISION. THE ASSAY TANKS ARE DIVIDED IN UPPER (B) AND LOWER (C) ZONE, BOTTOM (D), SURFACE (A) AND NOVEL OBJECT (E). THE NOVEL OBJECT CAN BE SEEN HANGING ABOVE THE SURFACE IN THE UPPER LEFT CORNER OF EACH ASSAY TANK.

The same behaviour in the different (NT versus NO) treatments were considered different behaviours in the statistical analysis due to the different contexts, creating in total ten behaviours that undergo investigation in this thesis.

The ten behaviours investigated (Table 3), were in both NT and NO treatments: activity measures, distance to surface, exploration measures and latency to enter the surface. All behaviours can be considered measures along a shy-bold continuum (Carter et al., 2013; Réale et al., 2010; Wright et al., 2019). Activity is the measure of number of body lengths moved by each individual fish per minute of each treatment. Following introduction into a new arena or tank, this behavioural measure is described as “exploration” (Carter et al., 2013). However, exploration is perhaps better understood as how much of the tank the fish has visited (e.g. Dingemanse et al., 2007). On the bold – shy continuum, the speed of exploration seems to be a more useful concept with shy individuals exploring more slowly and more extensively, with bold individuals exploring larger areas of the tank superficially as a result of heightened activity. Distance to the surface is the measure of the mean distance a fish keeps from the distance, in body lengths. A greater distance to surface might be seen as a measure of shy behaviour. Latency to enter the surface zone is calculated as the time in seconds before each individual fish first enters the surface zone, and again a larger value could be considered as shyer. All variables of latency were log transformed to ensure normal distributions of residual, and to more usefully allow proportional comparisons on differences in value. Distance to the NO is a measure of mean number of body lengths kept from the NO per minute per fish A large number indicated shy behaviour. In addition, a habituation measure was investigated in the NT treatment, where a small effect size would indicate boldness. The initial calculation of the habituation behaviour was conducted by Mette Helene Finnøen (PhD candidate), using the ancestral generation of the subject fish (F0 generation). This was done using segmented regression, to find the break point at which the slope of activity changes and flattens out in a NT treatment. This was found to be at ~6 min for both 26˚C and 30˚C, reflecting a rate at which the fish settled into their new surroundings (i.e. similar to other measures of ‘exploration’).

TABLE 3 OVERVIEW OF THE TEN BEHAVIOURS INVESTIGATED IN THIS PROJECT, WITH DESCRIPTION

Behaviour	Treatment	Description
Activity	NO	Distance moved total, in the novel tank treatment. Controlled for body lengths.
Activity	NT	Distance moved, total, in the novel object treatment. Controlled for body lengths
Distance to surface	NT	Mean distance to surface, in the novel tank treatment. Controlled for body lengths
Distance to surface	NO	Mean distance to surface, in the novel object treatment Controlled for body lengths
Latency to enter surface	NT	Latency to enter the surface for the first time, in the novel tank treatment
Latency to enter surface	NO	Latency to enter the surface for the first time, in the novel object treatment
Distance to NO	NO	Mean distance to NO. Controlled for body lengths
Habituation	NT	Slope of activity change the first 6 minutes of assay
Exploration	NT	How much of the tank that has been visited, in the novel tank treatment
Exploration	NO	How much of the tank that has been visited, in the novel object treatment

Statistical analysis in R

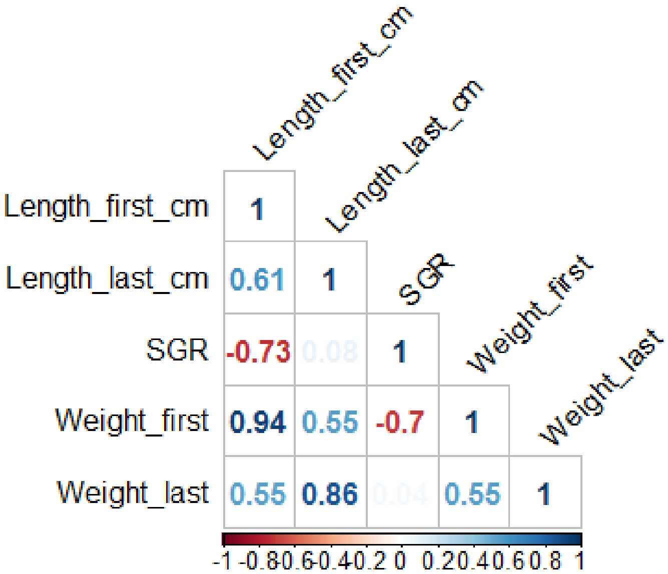
The statistical analysis was carried out using R 3.5.1 and R studio, and the packages Hmisc (Harrell Jr & Harrell Jr, 2019), car (Fox et al., 2012), lme4 (Bates et al., 2015), dplyr, a part of tidyverse (Wickham & Grolemund, 2016), ggplot2 (Wickham, 2016) and Segmented (Muggeo, 2008). Correlation matrices for both physiological data and behavioural data were produced using a Pearson correlation matrix.

Linear-mixed models were used to investigate the effect of temperature and order on all ten behaviours separately. To account for variation in behaviour among the different aquaria,

‘aquaria’ was used as a random factor in all linear-mixed models. Additionally, ‘fish identity’ was nested within ‘aquaria’ to account for multiple measures per fish. Model selection was performed based on Akaike information criterion (AIC) and significance values (Forstmeier & Schielzeth, 2011). Although there was a fixed effect of the ‘order’ of the temperatures, and this was therefore retained in final models, there were no significant (p=0.082) temperature-by-order interactions on any of the ten behaviours and this effect was therefore omitted from the final models. These univariate linear-mixed models were used to investigate the fixed effects selected line (high, low and random), temperature (26°C and 30°C) and order (1 or 2) on all ten behaviours separately. Temperature may have different effects on the three selected lines, and so an interaction between temperature and selected line was included.

The independent variables SGR and the first weight of the fish negatively covaried, and first and last weights positively covaried, but there was no relationship between last weight and SGR (Table 5). Therefore, all univariate tests for all ten behaviours included SGR and last weight, a model structure that was favoured by delta AIC values >2 compared to models including just first weight for all behaviours. Random is the control group, and hence the intercept used. Body lengths were controlled for in the activity, distance to surface and distance to the NO measures, by first calculating the mean lengths of each fish ((first weight + last weight)/2), and then dividing the behaviour in question with this individual length. These behaviours were initially registered in EthoVision in cm.

TABLE 4, PEARSON CORRELATION MATRIX, SHOWING THE CORRELATIONS OF SGR, FIRST AND LAST WEIGHT AND LENGTH. ON THIS BASIS, LENGTH WAS EXCLUDED FROM FURTHER ANALYSES, AS WEIGHT WAS CONSIDERED A BETTER MEASURE



	Length_first_cm	Length_last_cm	SGR	Weight_first	Weight_last
Length_first_cm	1				
Length_last_cm	0.61	1			
SGR	-0.73	0.08	1		
Weight_first	0.94	0.55	-0.7	1	
Weight_last	0.55	0.86	0.04	0.55	1

Ethical statement

Experiments were conducted in the Jutfelt Fish Ecophysiology Lab at NTNU, where Fredrik Jutfelt is PMSK. The experiment was approved by Norwegian Animal Research Authority (Permit Number: 8578). Animals were bred and kept at the institution, in approved facilities and in standard conditions (Reed & Jennings, 2011). Experiments were planned and executed with consideration of the three Rs in every step. The fish did not show signs of problematic effect of the behavioural assays, or the other treatments they were exposed to. At the end of all experiments, the fish were euthanized by approved methods for the lab (hypothermia). In total, only 12 fish died in during the experiments, which is ~5.5% (n=216), and two of these were euthanized in the early stages as they had a bent spine.

Results

Temperature and Line show no significance except for when they also show an effect in interaction (see Table 5)

As Table 5 and Figure 5 show, there were significant effects for the fixed effect interaction term **temperature*line** in many of the investigated behaviours, including activity in the NO treatment, distance to surface in the NO treatment, distance to NO, latency to enter the surface in the NT and NO treatments. The Low selected line was the most affected by temperature relative to the Random in four of these behaviours; increase in NO Activity, kept further away from the surface in the NO treatment, entered the surface more rapidly in the NT treatment, and kept a closer distance to the NO. Latency to enter the surface in the NO treatment was 0.98 sec faster in the elevated temperature for the High selected lines than the Random, whereas Low shows no effect of temperature for this behaviour. (For the complete summaries and anova table of all models, see appendix A)

The fixed effects of **SGR and weight** significantly affected 6 behaviours between them, the main trend being that bigger fish moved less, kept closer to the surface, explored more and moved closer to the NO (see Table 5). Faster growing fish kept a greater distance from the surface and moved less.

The fixed effect **order** of the assays was included in the models because it had an effect on all behaviours except latency to enter surface in the NO treatment, exploration in the NO treatment and perhaps unsurprisingly habituation. Interestingly, Habituation was not affected by any of the other fixed effects of temperature, line or size/growth (Table 5). As expected, during the second trial, fish kept closer to the NO, spent longer time before entering the surface, explored less, kept further away from the surface, showed less activity in the NO treatment and more activity in the NT treatment. All these results may indicate that the fish was more familiar with the set-up the second time around, and that the NT and NO were perhaps less "novel" (Table 5).

TABLE 5: RESULTS FOR LINEAR MIXED EFFECTS MODELS FOR EACH OF THE TEN INVESTIGATED BEHAVIOURS. DEGREES OF FREEDOM AND F VALUES ARE PROVIDED. SIGNIFICANT P VALUES ARE MARKED IN BOLD, AND THE EFFECT SIZES FOR THESE ARE PROVIDED.

	Activity NT			**Activity NO**		
	DF	F value	P value	DF	F value	P value
Line	2, 9.1	0.73	0.507	2, 9.2	0.70	0.520
Temperature 30	1, 7159.3	335.61	**<0.001**	1, 7154.2	0.42	0.519
Order B	1, 7157.5	144.33	**<0.001**	1, 7152.3	121.12	**<0.001**
SGR	1, 902.1	4.54	**0.033**	1, 908.2	4.01	0.045
Last weight	1, 340.3	3.42	0.065	1, 330.1	16.22	**<0.001**
*Line*Temperature*	2, 7158.7	0.57	0.568	2, 7153.7	15.43	**<0.001**
	Exploration NT			**Exploration NO**		
Line	2, 7.0	0.61	0.571	2, 8.8	3.54	0.074
Temperature 30	1, 175.3	2.79	0.097	1, 181.1	0.12	0.732
Order B	1, 176.0	8.06	**0.005**	1, 181.2	1.01	0.317
SGR	1, 94.1	0.13	0.724	1, 182.2	0.30	0.585
Last weight	1, 183.2	5.67	**0.018**	1, 181.9	0.37	0.545
*Line*Temperature*	2, 175.4	0.14	0.870	2, 181.2	0.06	0.944
	Latency to enter surface NT			**Latency to enter surface NO**		
Line	2, 9.2	0.68	0.532	2, 9.2	0.01	0.987
Temperature 30	1, 182.0	5.88	**0.016**	1, 180.6	7.81	**0.006**
Order B	1, 182.0	6.49	**0.012**	1, 180.6	0.12	0.726
SGR	1, 135.3	0.04	0.832	1, 195.0	0.47	0.495
Last weight	1, 186.8	1.56	0.213	1, 180.5	0.23	0.632
*Line*Temperature*	2, 182.0	3.69	**0.027**	2, 180.7	3.78	**0.025**

	Distance to surface NT			Distance to surface NO		
Line	2, 12.2	3.06	0.084	2, 12.1	0.85	0.452
Temperature 30	1, 7170.1	3.04	0.081	1, 7161.6	131.02	**<0.001**
Order B	1, 7168.7	31.96	**<0.001**	1,7159.8	70.50	**<0.001**
SGR	1, 473.1	0.31	0.580	1, 767.7	7.14	**0.008**
Last weight	1, 250.1	5.17	**0.024**	1, 288.5	3.23	0.073
*Line*Temperature*	2, 7169.9	2.31	0.099	2, 7161.1	13.06	**<0.001**
	Habituation			**Distance to NO**		
Line	2, 9.12	0.23	0.802	2, 8.7	6.68	**0.017**
Temperature 30	1, 181.4	1.01	0.317	1, 7195.3	42.22	**<0.001**
Order B	1, 181,4	0.25	0.618	1, 7193.5	110.71	**<0.001**
SGR	1, 170.7	1.20	0.274	1, 219.1	1.31	0.253
Last weight	1, 181.6	0.66	0.419	1, 354.1	27.03	**<0.001**
*Line*Temperature*	2, 181.4	0.82	0.440	2, 7194.7	30.92	**<0.001**

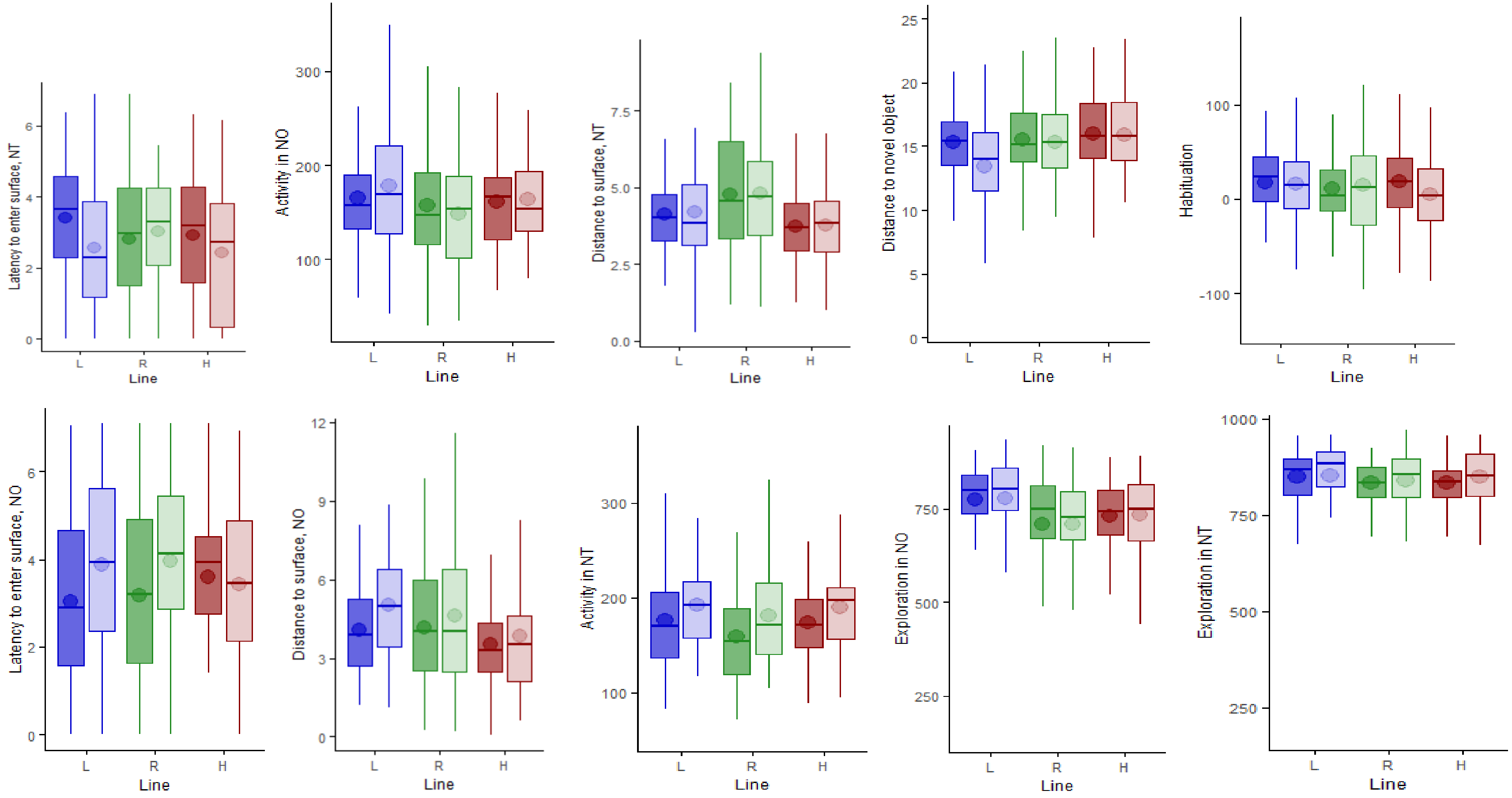


FIGURE 5, BOXPLOTS OF THE TEN BEHAVIOURS SPLIT BY LINE (BLUE=LOW, GREEN=RANDOM, RED=HIGH – SEE TEXT FOR DETAILS) AND TEMPERATURE (25°C=DARK, 30°C=LIGHT). BOLD LINES WITHIN EACH BOX REPRESENTS MEDIANS, DOTS REPRESENT MEANS, BOXES INDICATE VARIANCES, AND ERROR BARS THE 95% CIS

Covariances between the different behaviours

There were significant correlations between 25 of 45 pairs of behaviours – see Table 6. Interpreting the whole correlation matrix is difficult, but the trend seems to be that activity was important in these correlations between behaviours. Activity in NO and NT correlated with 11 of 15 possible behaviours (including each other). For details, see Table 6.

TABLE 6, PEARSON CORRELATIONS BETWEEN THE TEN DIFFERENT BEHAVIOURAL VARIABLES, WITH CORRELATION COEFFICIENTS AND P VALUES. SIGNIFICANT CORRELATIONS ARE MARKED IN BOLD AND CORRELATIONS OF THE SAME BEHAVIOUR IN DIFFERENT TREATMENTS ARE IN ITALICS.

Behaviour number	Hab	Act NO	Act NT	Exp NO	Exp NT	Lat NO	Lat NT	Dist to NO	Dist surf NO
Act NO	r= 0.05 p= 0.49								
Act NT	r= 0.03 p= 0.72	***r= 0.55*** ***p <0.001***							
Exp NO	r = -0.13 p= 0.08	**r= 0.49** **p <0.001**	**r= 0.33** **p= 0.02**						
Exp NT	**r= -0.17** **p= 0.02**	r= 0.13 p= 0.09	r= 0.76 p= 0.22	***r= 0.3*** ***p <0.001***					
Lat NO	r= -0.13 p= 0.09	**r= -0.39** **p <0.001**	**r= -0.26** **p <0.001**	**r= -0.15** **p= 0.04**	r= -0.06 p= 0.43				
Lat NT	**r= 0.2** **p= 0.01**	**r= -0.2** **p= 0.01**	**r= -0.18** **p= 0.02**	**r= -0.17** **p= 0.02**	r= -0.09 p= 0.23	***r= 0.29*** ***p <0.001***			
Dist to NO	r= 0.05 p= 0.52	r= -0.05 p= 0.52	r= -0.07 p= 0.36	**r= -0.25** **p <0.001**	r= 0.02 p= 0.76	r= -0.04 p= 0.62	r= 0.01 p= 0.88		
Dist surf NO	r= -0.05 p= 0.49	**r= -0.44** **p <0.001**	**r= -0.23** **p= 0.002**	r= -0.09 p= 0.21	r= -0.07 p= 0.35	**r= 0.39** **p <0.001**	**r= 0.29** **p <0.001**	**r= -0.42** **p <0.001**	
Dist surf NT	r= -0.14 p= 0.06	**r= -0.31** **p <0.001**	**r= 0.19** **p= 0.01**	r= -0.07 p= 0.34	**r= 0.53** **p <0.001**	**r= 0.19** **p= 0.01**	**r= 0.26** **p <0.001**	r= -0.08 p= 0.26	***r= 0.46*** ***p <0.001***

Discussion

The aim of this study was to test if a behavioural syndrome occurs and whether behaviours or changes in behaviour with temperature differed between lines of zebrafish selected for High, Random or Low CTmax performance. Physical attributes such as weight and growth rates (by length) were also investigated. Two possible hypotheses were presented to possibly explain any differences in behaviour. Firstly, that any syndrome is part of the aggression-boldness syndrome (Garamszegi et al., 2012) and possibly reflecting a wider pace-of-life syndrome (see Wright et al., 2019) and secondly that selection for high CTmax was in fact selection on high quality individuals meaning that low CTmax individual would exhibit a lack of genetic and/or phenotypic 'quality' in various aspects of their behaviour.

For hypothesis 1, the most important prediction is the differences in the mean behaviours between the lines i.e. if L line is consistently shy and H is consistently bold. However, no such differences were found. Neither was there any particular evidence for POLS in the physiological measures, even if SGR and body weight affected six of the ten behaviours between them. The POLS theory suggests that there could be a link between physiological traits, life history traits and behavioural traits (see Réale et al., 2010). Bigger and more rapidly growing fish should be bolder, in the sense that they should be more active, keep closer to the surface and NO, and have a shorter latency to enter the surface, which were only true for three behaviours (for details, see appendix A, summaries with effect sizes), suggesting that the direction of these results are arbitrary. The Line by Temperature interaction showed that behaviour in the Low selected line was often more affected by temperature differences than the Random lines. From a POLS theory point of view, the Low line could be able to cope with these different temperatures by having a larger behavioural plasticity, and hence this is the strongest argument that there is evidence supporting POLS theory. For hypothesis 2, there may be stronger evidence in the results presented here, and differences are visible between lines – especially in effect of elevated temperature. Even though this plasticity to cope with temperature change may be a valuable trait, it may also be a consequence of less physiological or biochemical plasticity. This would mean that the L line is less able to cope with ambient temperature change, and the behavioural plasticity is a mere symptom of this. This could indicate that selection on lower CTmax included selection for poorer quality individuals overall, whereas selection on high CTmax tolerance was selection on higher quality individuals (with some sort of upper CTmax limit for tolerance – see Fig. B1 in Appendix B). The notion being that everything in the body must be functioning well for an individual to tolerate high temperatures, whereas many things can go wrong to produce an individual unable to cope with high temperatures. This idea is also supported by the CTmax versus CTmin (critical thermal minimum) performances of these same fish from the masters project by Hildrum (2019), which show that individuals with a low CTmax also showed a higher CTmin, and that the individuals with a high CTmax also had a lower CTmin, from the L and H lines, respectively.

A within-individual analysis of the results from Hildrum's (2019) and the results from this experiment would be interesting to look at as a future research project, to obtain more possible evidence for either hypotheses. Also, structural equation modelling (SEM) could be used to further investigate the behaviours, body measures and their correlations tested in this experiment.

Concluding remarks

Together, the results presented here perhaps indicate stronger support for hypothesis 2; selection on individuals with a high CTmax seems to have constituted selection for high-quality zebrafish in general. They were more able to produce consistent and less altered behaviours following temperature changes (26˚C to 30˚C), that is hard to place on the shy - bold continuum of POLS behavioural traits (Carter et al., (2013); Réale et al., (2010); Wright et al. (2019)).

This study shows that selection on CTmax also affects behavioural traits and the scope for coping with different temperatures. Selection on high CTmax does not appear to produce a particularly bold vs shy behavioural syndrome, supporting POLS theory. However, useful first evidence was found for selection on thermal tolerance also means selection on covarying behaviours and behavioural plastic responses to different temperatures.

References

Andrew Sih, Alison M Bell, J Chadwick Johnson, & Robert E Ziemba. (2004). Behavioral Syndromes: An Integrative Overview. *The Quarterly Review of Biology, 79*(3), 241-277. doi:10.1086/422893

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., . . . Rcpp, L. (2015). Package 'lme4'. *Convergence, 12*(1).

Bennett, A. F. (1980). The thermal dependence of lizard behaviour. *Animal Behaviour, 28*(3), 752-762.

Biro, P. A., Beckmann, C., & Stamps, J. A. (2009). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proc Biol Sci, 277*(1678), 71-77.

Brodie III, E., & Russell, N. H. (1999). The consistency of individual differences in behaviour: temperature effects on antipredator behaviour in garter snakes. *Animal Behaviour, 57*(2), 445-451.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology, 85*(7), 1771-1789.

Careau, V., Thomas, D., Humphries, M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos, 117*(5), 641-653.

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological reviews, 88*(2), 465-475.

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1560), 4021-4028.

Dingemanse, N. J., Both, C., Van Noordwijk, A. J., Rutten, A. L., & Drent, P. J. (2003). Natal dispersal and personalities in great tits (Parus major). *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1516), 741-747.

Dingemanse, N. J., Dochtermann, N., & Wright, J. (2010b). A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Animal Behaviour, 79*(2), 439-450.

Dingemanse, N. J., Kazem, A. J., Réale, D., & Wright, J. (2010a). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution, 25*(2), 81-89.

Forstmeier, W., & Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. *Behavioral Ecology and Sociobiology, 65*(1), 47-55.

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., . . . Graves, S. (2012). Package 'car'. *Vienna: R Foundation for Statistical Computing*.

Garamszegi, L.Z., Marko, G. & Herczeg, G. (2012) A meta-analysis of correlated behaviours with implications for behavioural syndromes: mean effect size, publication bias, phylogenetic effects and the role of mediator variables. *Evol. Ecol.* 26, 1213-1235.

Harrell Jr, F. E., & Harrell Jr, M. F. E. (2019). Package 'Hmisc'. *CRAN2018*, 235-236.

Hildrum, Hanne, 2019, "Selection on high thermal tolerance alters tolerance to cold" NTNU

Kern, E. M. A., Robinson, D., Gass, E., Godwin, J., & Langerhans, R. B. (2016). Correlated evolution of personality, morphology and performance. *Animal Behaviour, 117*, 79-86. doi:https://doi.org/10.1016/j.anbehav.2016.04.007

López-Olmeda, J. F., & Sánchez-Vázquez, F. J. (2011). Thermal biology of zebrafish (Danio rerio). *Journal of Thermal Biology, 36*(2), 91-104. doi:https://doi.org/10.1016/j.jtherbio.2010.12.005

Mitchell, D. J., & Biro, P. A. (2017). Is behavioural plasticity consistent across different environmental gradients and through time? *Proc Biol Sci, 284*(1860), 20170893.

Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CTmax is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports, 8*(1), 7099. doi:10.1038/s41598-018-25593-4

Muggeo, V. M. (2008). Segmented: an R package to fit regression models with broken-line relationships. *R news, 8*(1), 20-25.

Noldus, 2019, © 2019 Noldus Information Technology retrieved at: https://www.noldus.com/animal-behavior-research/products/ethovision-xt, 21.05.2019

Nusslein-Volhard, C., & Dahm, R. (2002). *Zebrafish*: Oxford University Press.

Pang, X., Fu, S.-J., & Zhang, Y.-G. (2017). Individual variation in metabolism and thermal tolerance in juvenile qingbo (Spinibarbus sinensis). *Marine and Freshwater Behaviour and Physiology, 50*(5-6), 359-373. doi:10.1080/10236244.2017.1416269

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P.-O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 365*(1560), 4051-4063.

Reed, B., & Jennings, M. (2011). Guidance on the housing and care of Zebrafish. *Southwater: Royal Society for the Prevention of Cruelty to Animals*.

Rey, S., Digka, N., & MacKenzie, S. (2015). Animal personality relates to thermal preference in wild-type zebrafish, Danio rerio. *Zebrafish, 12*(3), 243-249.

Roy, T., & Bhat, A. (2018). Population, sex and body size: determinants of behavioural variations and behavioural correlations among wild zebrafish Danio rerio. *Royal Society open science, 5*(1), 170978.

Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology, 51*(5), 691-702. doi:10.1093/icb/icr097

Seneviratne, S. I., Donat, M. G., Mueller, B., & Alexander, L. V. (2014). No pause in the increase of hot temperature extremes. *Nature Climate Change, 4*(3), 161.

Spence, R., Fatema, M., Ellis, S., Ahmed, Z., & Smith, C. (2007). Diet, growth and recruitment of wild zebrafish in Bangladesh. *Journal of Fish Biology, 71*(1), 304-309.

Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, Danio rerio. *Biological reviews, 83*(1), 13-34.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*: Springer.

Wickham, H., & Grolemund, G. (2016). *R for data science: import, tidy, transform, visualize, and model data*: " O'Reilly Media, Inc.".

Wisenden, B. D., Sailer, C. D., Radenic, S. J., & Sutrisno, R. (2011). Maternal inheritance and exploratory-boldness behavioural syndrome in zebrafish. *Behaviour*, 1443-1456.

Wong, R. Y., Perrin, F., Oxendine, S. E., Kezios, Z. D., Sawyer, S., Zhou, L., . . . Godwin, J. (2012). Comparing behavioral responses across multiple assays of stress and anxiety in zebrafish (Danio rerio). *Behaviour, 149*(10), 1205.

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1549), 2093-2106.

Wright, J., Bolstad, G. H., Araya-Ajoy, Y. G., & Dingemanse, N. J. (2019). Life-history evolution under fluctuating density-dependent selection and the adaptive alignment of pace-of-life syndromes. *Biological reviews, 94*(1), 230-247.

Zhang, Y., & Kieffer, J. D. (2014). Critical thermal maximum (CTmax) and hematology of shortnose sturgeons (Acipenser brevirostrum) acclimated to three temperatures. *Canadian Journal of Zoology, 92*(3), 215-221.

Appendices

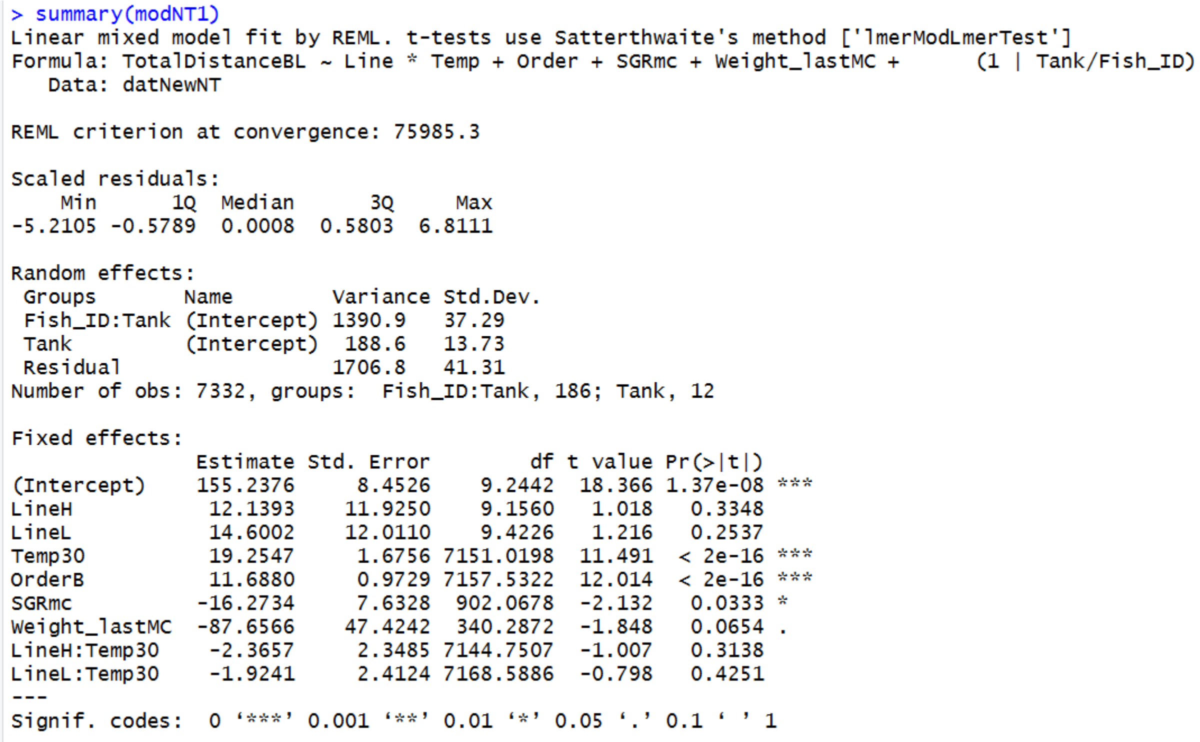
Appendix A, summaries and anovas of linear models for behaviours

Activity summary and anova table

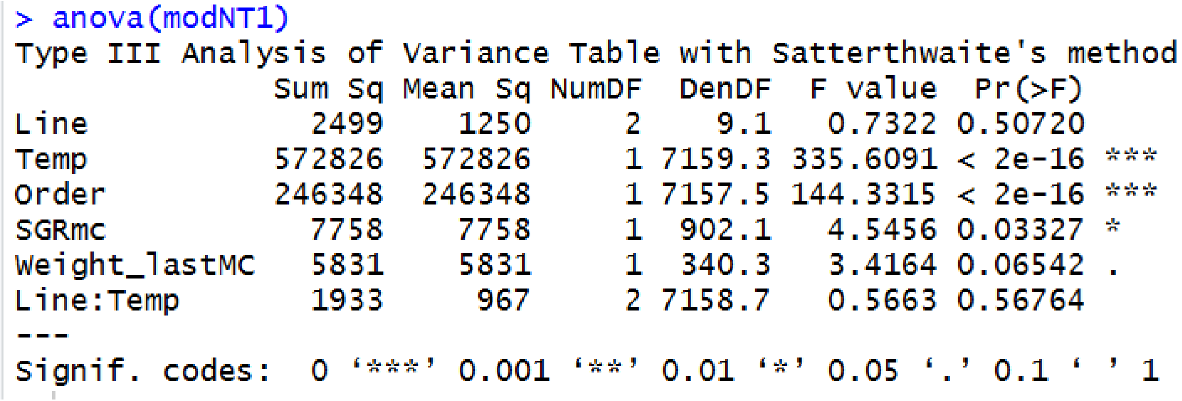
Novel tank treatment

modNT1 <- lmer(TotalDistanceBL ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNT, REML = T)



```
> summary(modNT1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: TotalDistanceBL ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNT

REML criterion at convergence: 75985.3

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-5.2105 -0.5789  0.0008  0.5803  6.8111 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 1390.9   37.29   
 Tank         (Intercept)  188.6   13.73   
 Residual                 1706.8   41.31   
Number of obs: 7332, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
               Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)    155.2376     8.4526    9.2442  18.366 1.37e-08 ***
LineH           12.1393    11.9250    9.1560   1.018   0.3348    
LineL           14.6002    12.0110    9.4226   1.216   0.2537    
Temp30          19.2547     1.6756 7151.0198  11.491  < 2e-16 ***
OrderB          11.6880     0.9729 7157.5322  12.014  < 2e-16 ***
SGRmc          -16.2734     7.6328  902.0678  -2.132   0.0333 *  
Weight_lastMC  -87.6566    47.4242  340.2872  -1.848   0.0654 .  
LineH:Temp30    -2.3657     2.3485 7144.7507  -1.007   0.3138    
LineL:Temp30    -1.9241     2.4124 7168.5886  -0.798   0.4251    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



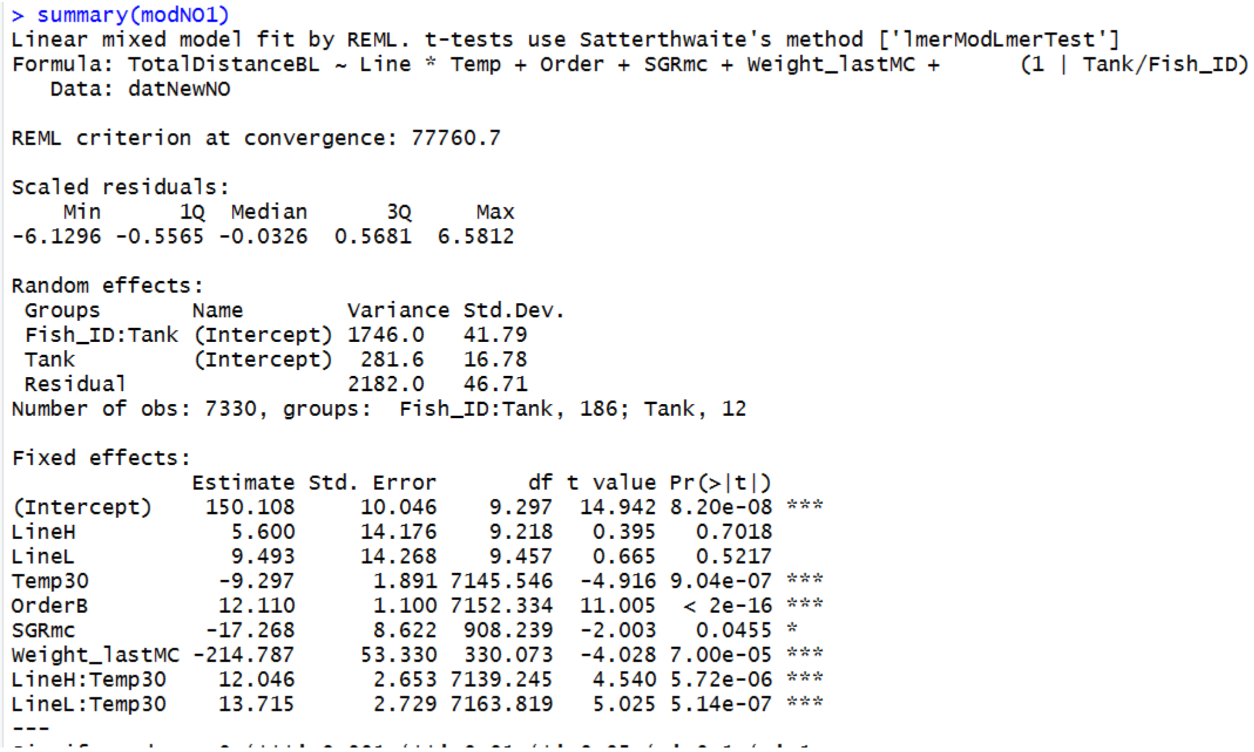
```
> anova(modNT1)
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF  F value  Pr(>F)    
Line            2499    1250     2    9.1   0.7322 0.50720    
Temp          572826  572826     1 7159.3 335.6091 < 2e-16 ***
Order         246348  246348     1 7157.5 144.3315 < 2e-16 ***
SGRmc           7758    7758     1  902.1   4.5456 0.03327 *  
Weight_lastMC   5831    5831     1  340.3   3.4164 0.06542 .  
Line:Temp       1933     967     2 7158.7   0.5663 0.56764    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Activity summary and anova table

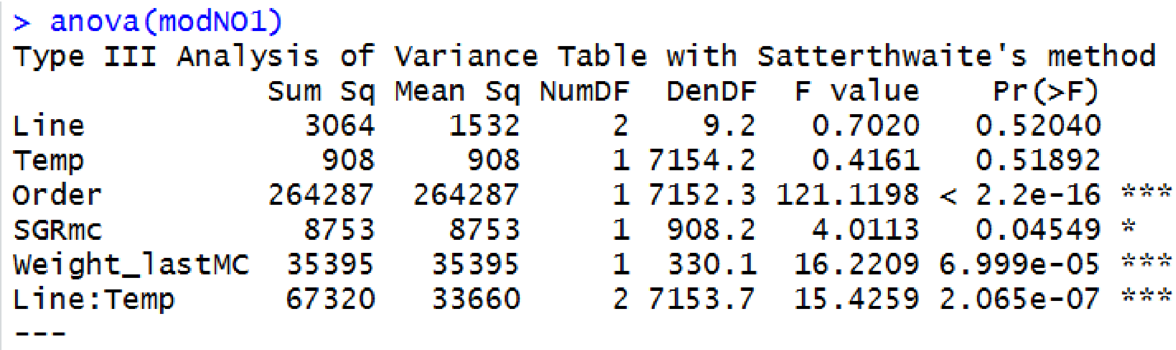
Novel object treatment

modNO1 <- lmer(TotalDistanceBL ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNO, REML = T)



```
> summary(modNO1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: TotalDistanceBL ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNO

REML criterion at convergence: 77760.7

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-6.1296 -0.5565 -0.0326  0.5681  6.5812 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 1746.0   41.79   
 Tank         (Intercept)  281.6   16.78   
 Residual                 2182.0   46.71   
Number of obs: 7330, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
              Estimate Std. Error       df t value Pr(>|t|)    
(Intercept)    150.108     10.046    9.297  14.942 8.20e-08 ***
LineH            5.600     14.176    9.218   0.395   0.7018    
LineL            9.493     14.268    9.457   0.665   0.5217    
Temp30          -9.297      1.891 7145.546  -4.916 9.04e-07 ***
OrderB          12.110      1.100 7152.334  11.005  < 2e-16 ***
SGRmc          -17.268      8.622  908.239  -2.003   0.0455 *  
Weight_lastMC -214.787     53.330  330.073  -4.028 7.00e-05 ***
LineH:Temp30    12.046      2.653 7139.245   4.540 5.72e-06 ***
LineL:Temp30    13.715      2.729 7163.819   5.025 5.14e-07 ***
---
```



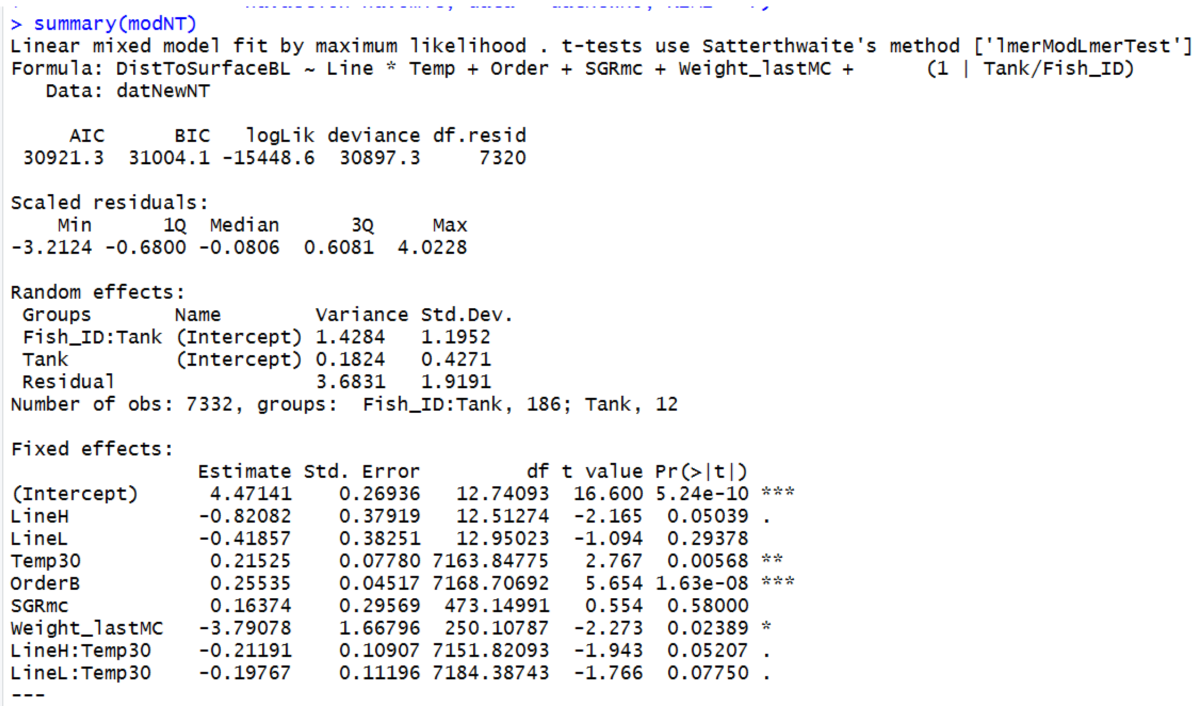
```
> anova(modNO1)
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF  F value    Pr(>F)    
Line            3064    1532     2    9.2   0.7020   0.52040    
Temp             908     908     1 7154.2   0.4161   0.51892    
Order         264287  264287     1 7152.3 121.1198 < 2.2e-16 ***
SGRmc           8753    8753     1  908.2   4.0113   0.04549 *  
Weight_lastMC  35395   35395     1  330.1  16.2209 6.999e-05 ***
Line:Temp      67320   33660     2 7153.7  15.4259 2.065e-07 ***
---
```

Distance to surface summary and anova table

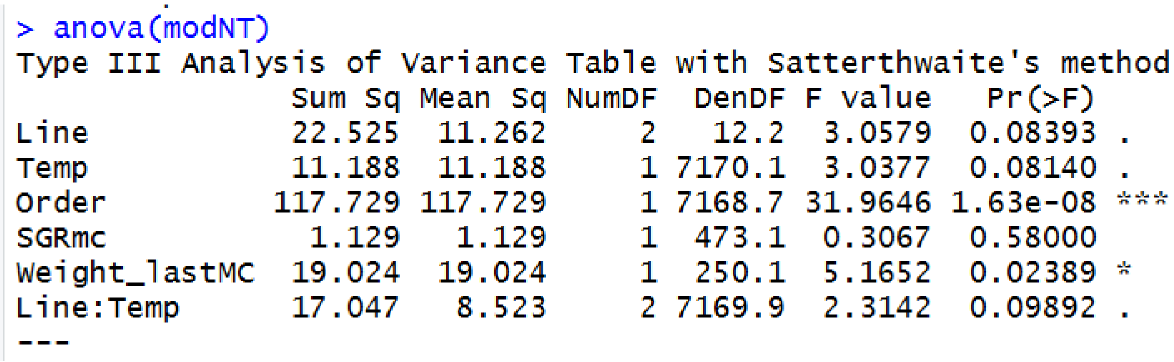
Novel tank treatment

modNT <- lmer(DistToSurfaceBL ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNT, REML = F)



```
> summary(modNT)
Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: DistToSurfaceBL ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNT

     AIC      BIC   logLik deviance df.resid 
 30921.3  31004.1 -15448.6  30897.3     7320 

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-3.2124 -0.6800 -0.0806  0.6081  4.0228 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 1.4284   1.1952  
 Tank         (Intercept) 0.1824   0.4271  
 Residual                 3.6831   1.9191  
Number of obs: 7332, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
                Estimate Std. Error         df t value Pr(>|t|)    
(Intercept)      4.47141    0.26936   12.74093  16.600 5.24e-10 ***
LineH           -0.82082    0.37919   12.51274  -2.165  0.05039 .  
LineL           -0.41857    0.38251   12.95023  -1.094  0.29378    
Temp30           0.21525    0.07780 7163.84775   2.767  0.00568 ** 
OrderB           0.25535    0.04517 7168.70692   5.654 1.63e-08 ***
SGRmc            0.16374    0.29569  473.14991   0.554  0.58000    
Weight_lastMC   -3.79078    1.66796  250.10787  -2.273  0.02389 *  
LineH:Temp30    -0.21191    0.10907 7151.82093  -1.943  0.05207 .  
LineL:Temp30    -0.19767    0.11196 7184.38743  -1.766  0.07750 .  
---
```



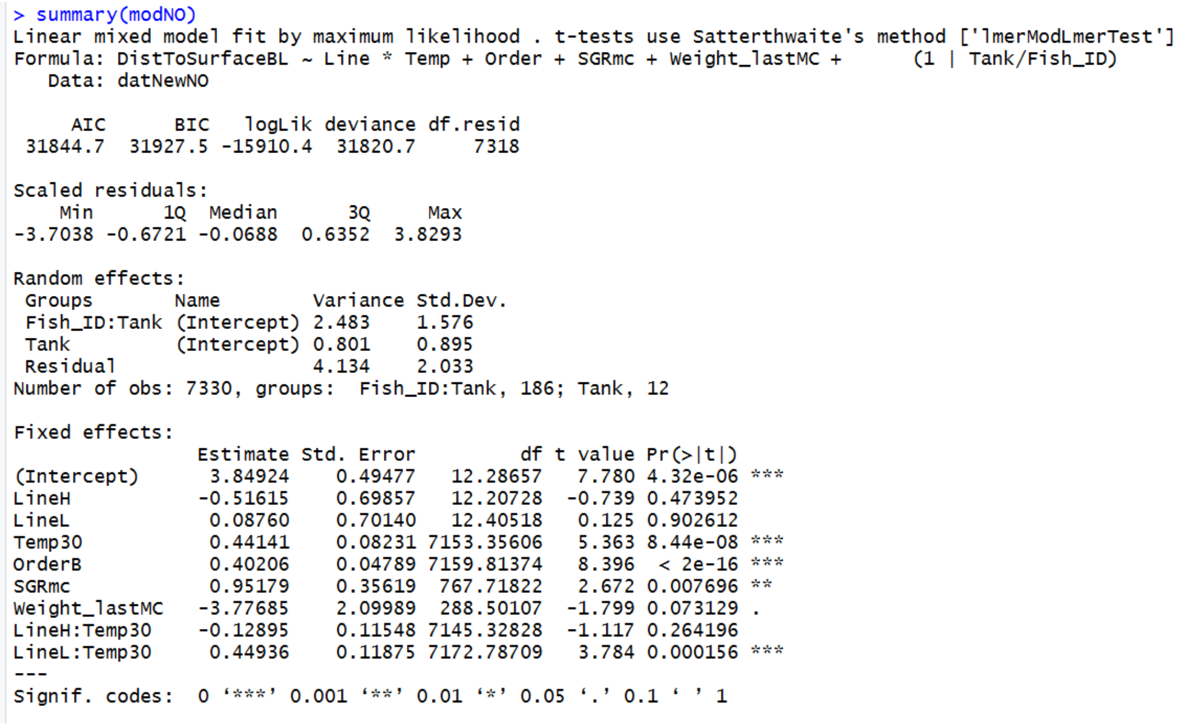
```
> anova(modNT)
Type III Analysis of Variance Table with Satterthwaite's method
               Sum Sq Mean Sq NumDF  DenDF F value   Pr(>F)    
Line           22.525  11.262     2   12.2  3.0579  0.08393 .  
Temp           11.188  11.188     1 7170.1  3.0377  0.08140 .  
Order         117.729 117.729     1 7168.7 31.9646 1.63e-08 ***
SGRmc           1.129   1.129     1  473.1  0.3067  0.58000    
Weight_lastMC  19.024  19.024     1  250.1  5.1652  0.02389 *  
Line:Temp      17.047   8.523     2 7169.9  2.3142  0.09892 .  
---
```

Distance to surface summary and anova table

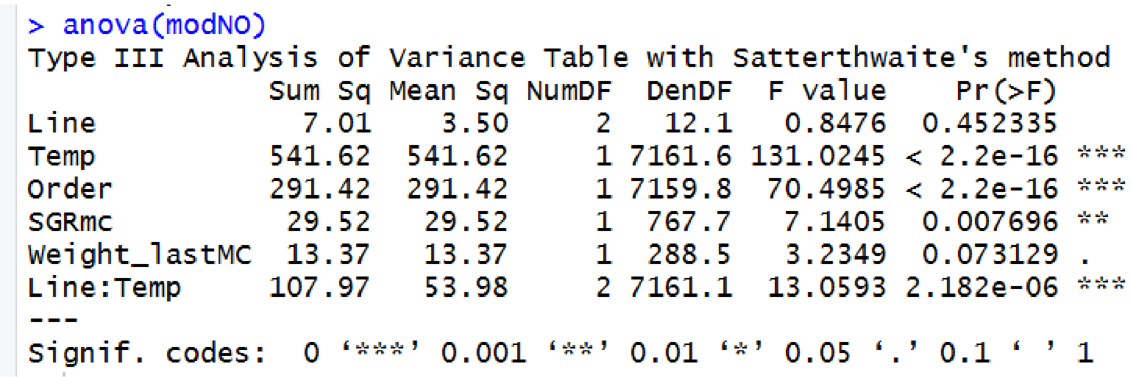
Novel object treatment

modNO <- lmer(DistToSurfaceBL ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNO, REML = F)



```
> summary(modNO)
Linear mixed model fit by maximum likelihood . t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: DistToSurfaceBL ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNO

     AIC      BIC   logLik deviance df.resid 
 31844.7  31927.5 -15910.4  31820.7     7318 

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-3.7038 -0.6721 -0.0688  0.6352  3.8293 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 2.483    1.576   
 Tank         (Intercept) 0.801    0.895   
 Residual                 4.134    2.033   
Number of obs: 7330, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
               Estimate Std. Error         df t value Pr(>|t|)    
(Intercept)     3.84924    0.49477   12.28657   7.780 4.32e-06 ***
LineH          -0.51615    0.69857   12.20728  -0.739 0.473952    
LineL           0.08760    0.70140   12.40518   0.125 0.902612    
Temp30          0.44141    0.08231 7153.35606   5.363 8.44e-08 ***
OrderB          0.40206    0.04789 7159.81374   8.396  < 2e-16 ***
SGRmc           0.95179    0.35619  767.71822   2.672 0.007696 ** 
Weight_lastMC  -3.77685    2.09989  288.50107  -1.799 0.073129 .  
LineH:Temp30   -0.12895    0.11548 7145.32828  -1.117 0.264196    
LineL:Temp30    0.44936    0.11875 7172.78709   3.784 0.000156 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



```
> anova(modNO)
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF  F value    Pr(>F)    
Line            7.01    3.50     2   12.1   0.8476  0.452335    
Temp          541.62  541.62     1 7161.6 131.0245 < 2.2e-16 ***
Order         291.42  291.42     1 7159.8  70.4985 < 2.2e-16 ***
SGRmc          29.52   29.52     1  767.7   7.1405  0.007696 ** 
Weight_lastMC  13.37   13.37     1  288.5   3.2349  0.073129 .  
Line:Temp     107.97   53.98     2 7161.1  13.0593 2.182e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

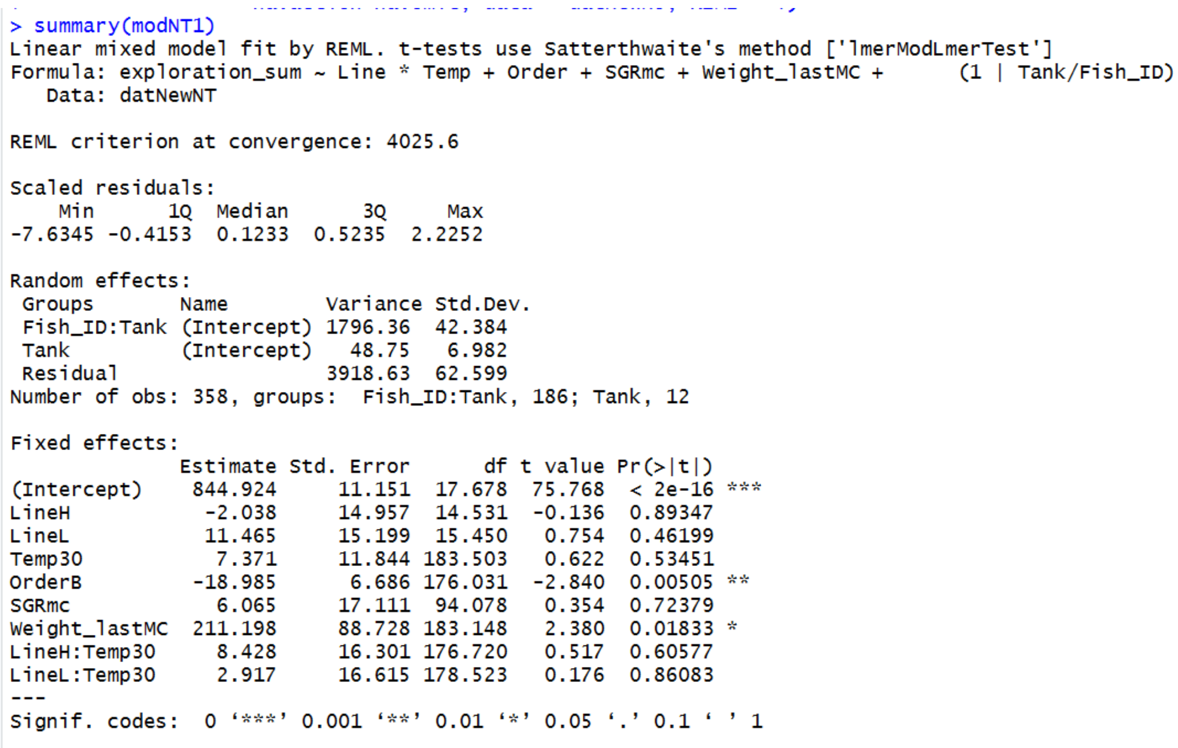
Exploration summary and anova table

Novel tank treatment

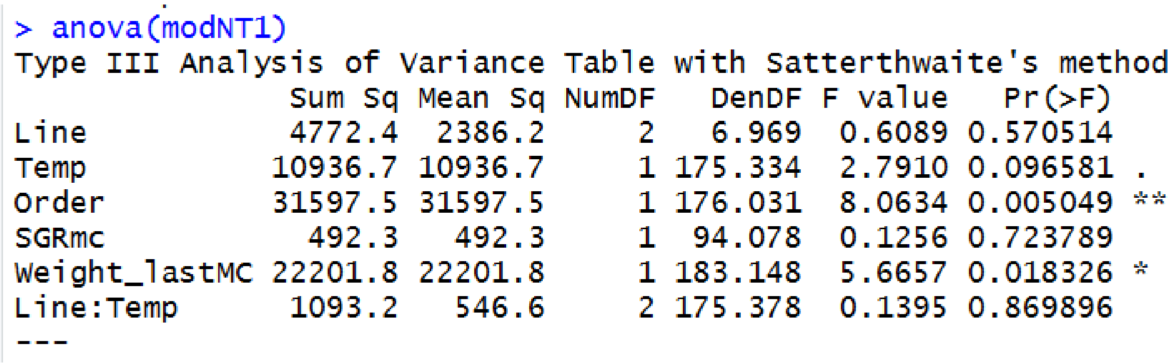
modNT1 <- lmer(exploration_sum ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNT, REML = T)

model without singular fit and convergence problems.



```
> summary(modNT1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: exploration_sum ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNT

REML criterion at convergence: 4025.6

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-7.6345 -0.4153  0.1233  0.5235  2.2252 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 1796.36  42.384  
 Tank         (Intercept)   48.75   6.982  
 Residual                 3918.63  62.599  
Number of obs: 358, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
              Estimate Std. Error      df t value Pr(>|t|)    
(Intercept)    844.924     11.151  17.678  75.768  < 2e-16 ***
LineH           -2.038     14.957  14.531  -0.136  0.89347    
LineL           11.465     15.199  15.450   0.754  0.46199    
Temp30           7.371     11.844 183.503   0.622  0.53451    
OrderB         -18.985      6.686 176.031  -2.840  0.00505 ** 
SGRmc            6.065     17.111  94.078   0.354  0.72379    
Weight_lastMC  211.198     88.728 183.148   2.380  0.01833 *  
LineH:Temp30     8.428     16.301 176.720   0.517  0.60577    
LineL:Temp30     2.917     16.615 178.523   0.176  0.86083    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



```
> anova(modNT1)
Type III Analysis of Variance Table with Satterthwaite's method
               Sum Sq Mean Sq NumDF   DenDF F value   Pr(>F)   
Line           4772.4  2386.2     2   6.969  0.6089 0.570514   
Temp          10936.7 10936.7     1 175.334  2.7910 0.096581 . 
Order         31597.5 31597.5     1 176.031  8.0634 0.005049 **
SGRmc           492.3   492.3     1  94.078  0.1256 0.723789   
Weight_lastMC 22201.8 22201.8     1 183.148  5.6657 0.018326 * 
Line:Temp      1093.2   546.6     2 175.378  0.1395 0.869896   
---
```

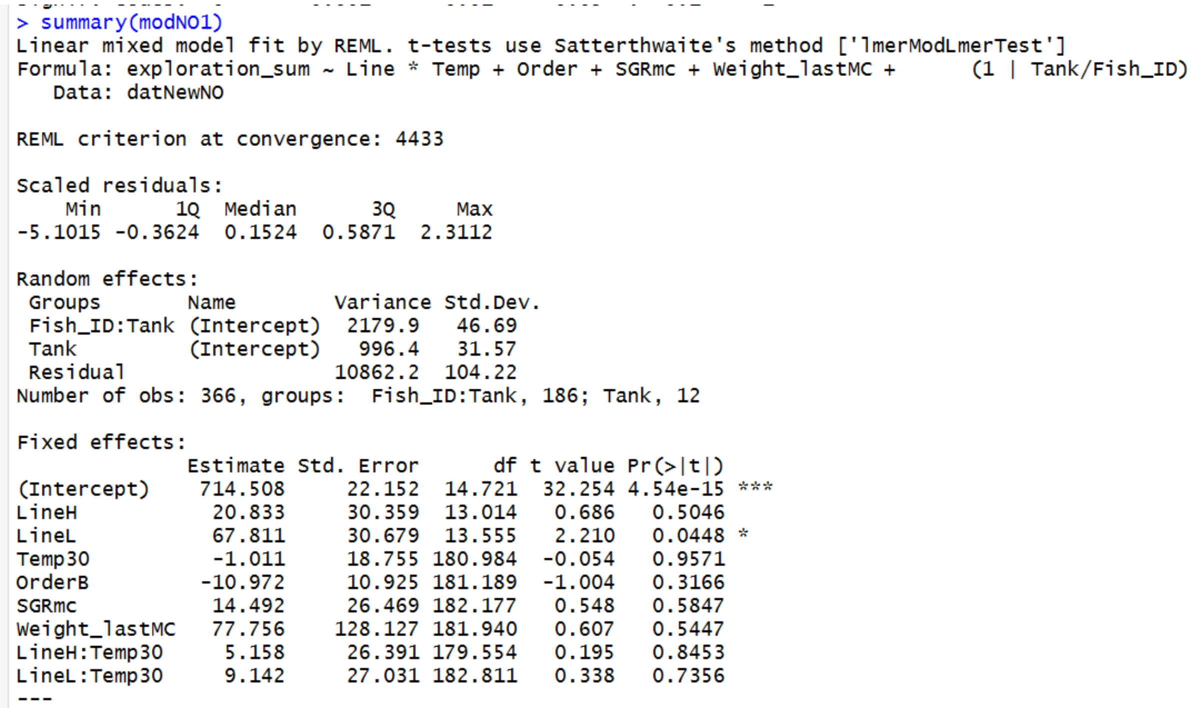
Exploration summary and anova table

Novel object treatment

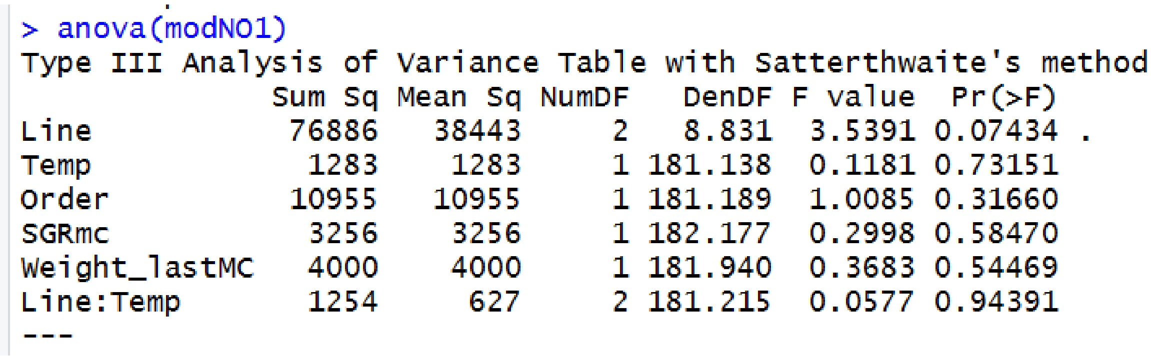
modNO1 <- lmer(exploration_sum ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNO, REML = T)

model without singular fit and convergence problems.



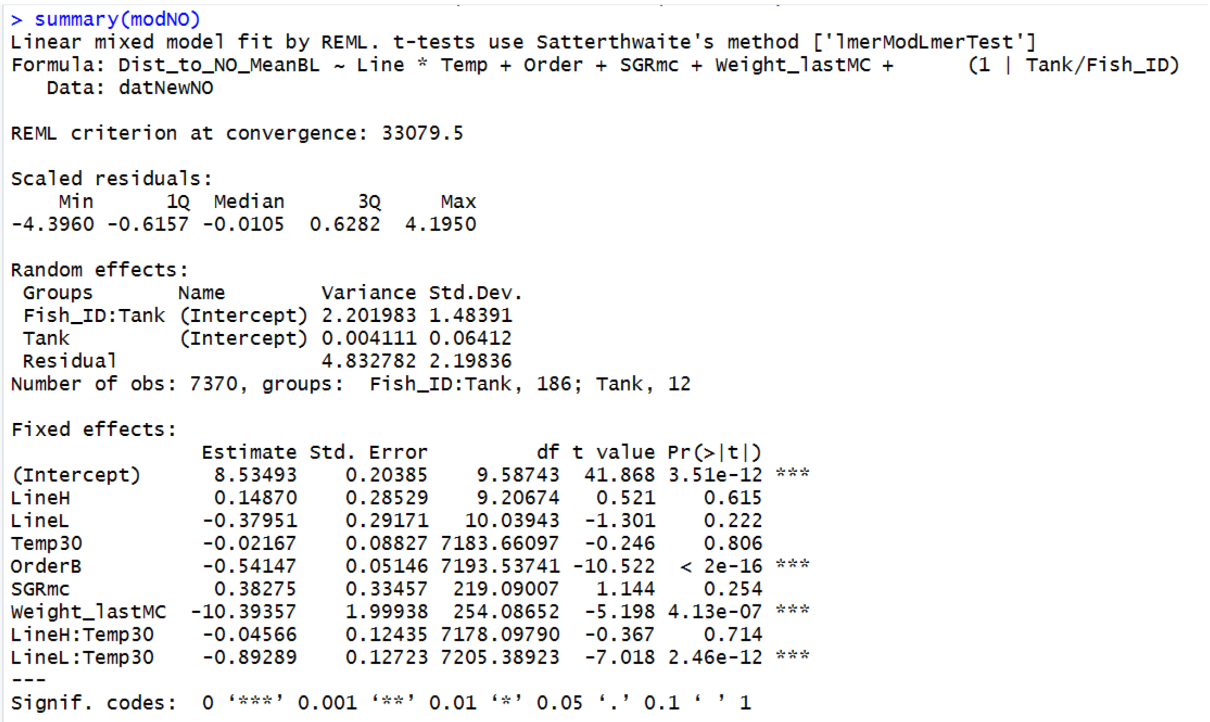
```
> summary(modNO1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: exploration_sum ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNO

REML criterion at convergence: 4433

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-5.1015 -0.3624  0.1524  0.5871  2.3112 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept)  2179.9   46.69  
 Tank         (Intercept)   996.4   31.57  
 Residual                 10862.2  104.22  
Number of obs: 366, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
              Estimate Std. Error      df t value Pr(>|t|)    
(Intercept)    714.508     22.152  14.721  32.254 4.54e-15 ***
LineH           20.833     30.359  13.014   0.686   0.5046    
LineL           67.811     30.679  13.555   2.210   0.0448 *  
Temp30          -1.011     18.755 180.984  -0.054   0.9571    
OrderB         -10.972     10.925 181.189  -1.004   0.3166    
SGRmc           14.492     26.469 182.177   0.548   0.5847    
Weight_lastMC   77.756    128.127 181.940   0.607   0.5447    
LineH:Temp30     5.158     26.391 179.554   0.195   0.8453    
LineL:Temp30     9.142     27.031 182.811   0.338   0.7356    
---
```



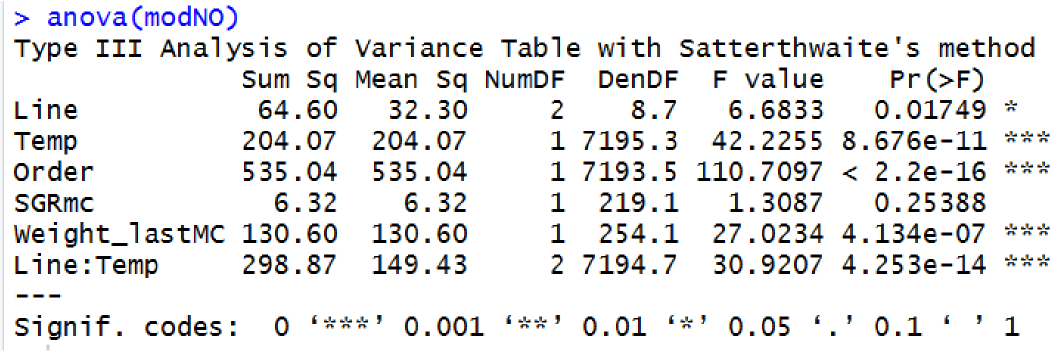
```
> anova(modNO1)
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF   DenDF F value  Pr(>F)  
Line           76886   38443     2   8.831  3.5391 0.07434 .
Temp            1283    1283     1 181.138  0.1181 0.73151  
Order          10955   10955     1 181.189  1.0085 0.31660  
SGRmc           3256    3256     1 182.177  0.2998 0.58470  
Weight_lastMC   4000    4000     1 181.940  0.3683 0.54469  
Line:Temp       1254     627     2 181.215  0.0577 0.94391  
---
```

Distance to novel object summary and anova table

modNO <- lmer(Dist_to_NO_MeanBL ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID), na.action=na.omit, data = datNewNO, REML = T)

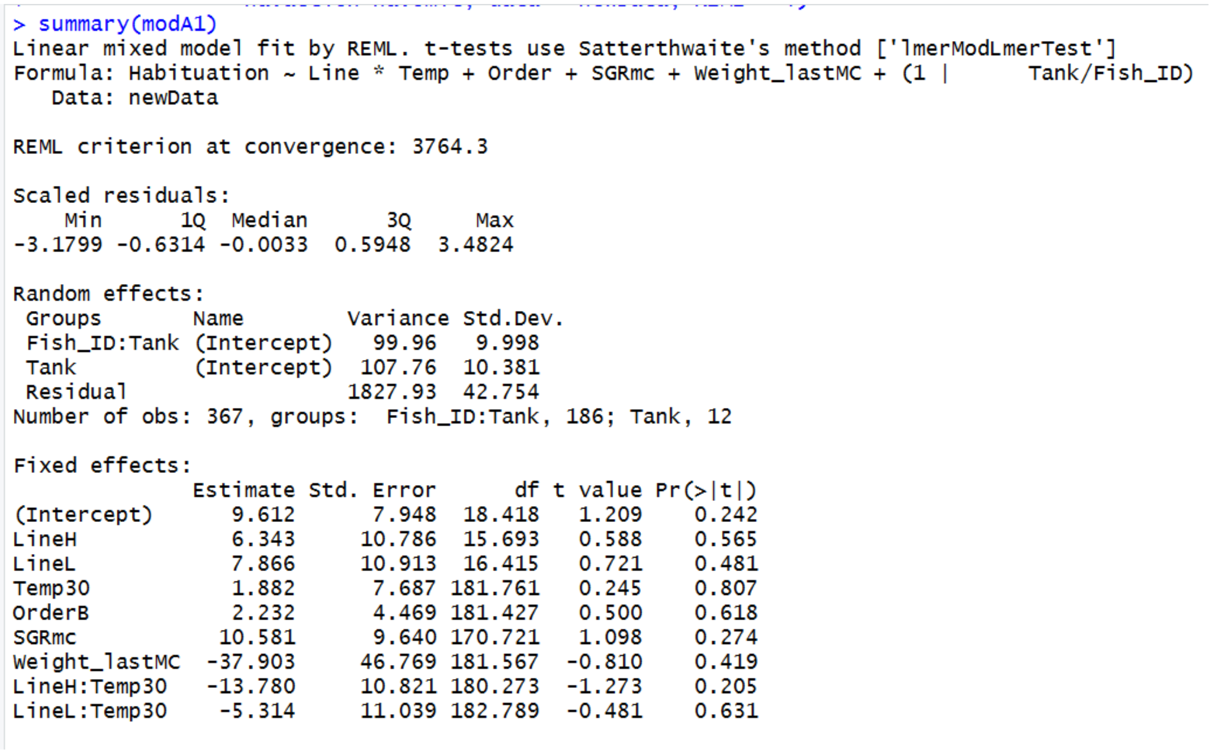


```
> summary(modNO)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: Dist_to_NO_MeanBL ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNO

REML criterion at convergence: 33079.5

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-4.3960 -0.6157 -0.0105  0.6282  4.1950 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 2.201983 1.48391 
 Tank         (Intercept) 0.004111 0.06412 
 Residual                 4.832782 2.19836 
Number of obs: 7370, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
                Estimate Std. Error         df t value Pr(>|t|)    
(Intercept)      8.53493    0.20385    9.58743  41.868 3.51e-12 ***
LineH            0.14870    0.28529    9.20674   0.521    0.615    
LineL           -0.37951    0.29171   10.03943  -1.301    0.222    
Temp30          -0.02167    0.08827 7183.66097  -0.246    0.806    
OrderB          -0.54147    0.05146 7193.53741 -10.522  < 2e-16 ***
SGRmc            0.38275    0.33457  219.09007   1.144    0.254    
Weight_lastMC  -10.39357    1.99938  254.08652  -5.198 4.13e-07 ***
LineH:Temp30    -0.04566    0.12435 7178.09790  -0.367    0.714    
LineL:Temp30    -0.89289    0.12723 7205.38923  -7.018 2.46e-12 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

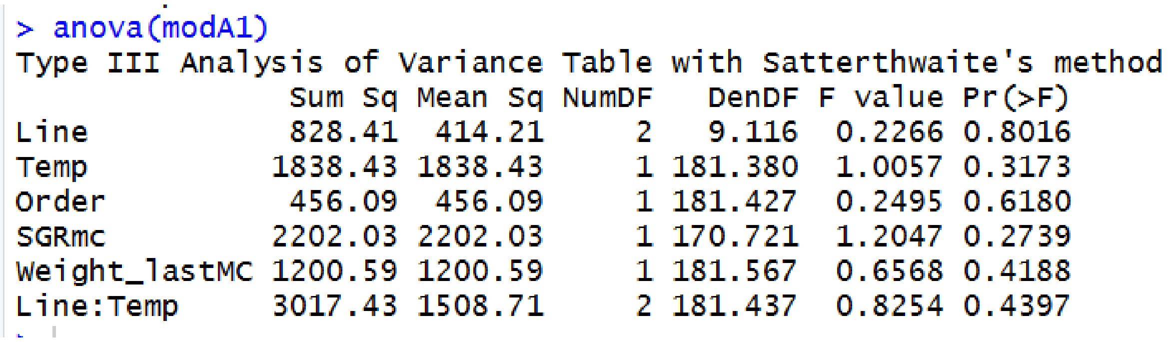


```
> anova(modNO)
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF  DenDF  F value    Pr(>F)    
Line           64.60   32.30     2    8.7   6.6833   0.01749 *  
Temp          204.07  204.07     1 7195.3  42.2255 8.676e-11 ***
Order         535.04  535.04     1 7193.5 110.7097 < 2.2e-16 ***
SGRmc           6.32    6.32     1  219.1   1.3087   0.25388    
Weight_lastMC 130.60  130.60     1  254.1  27.0234 4.134e-07 ***
Line:Temp     298.87  149.43     2 7194.7  30.9207 4.253e-14 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Habituation summary and anova table



```
> summary(modA1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: Habituation ~ Line * Temp + Order + SGRmc + Weight_lastMC + (1 |       Tank/Fish_ID)
   Data: newData

REML criterion at convergence: 3764.3

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-3.1799 -0.6314 -0.0033  0.5948  3.4824 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept)   99.96   9.998  
 Tank         (Intercept)  107.76  10.381  
 Residual                 1827.93  42.754  
Number of obs: 367, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
             Estimate Std. Error      df t value Pr(>|t|)
(Intercept)     9.612      7.948  18.418   1.209    0.242
LineH           6.343     10.786  15.693   0.588    0.565
LineL           7.866     10.913  16.415   0.721    0.481
Temp30          1.882      7.687 181.761   0.245    0.807
OrderB          2.232      4.469 181.427   0.500    0.618
SGRmc          10.581      9.640 170.721   1.098    0.274
Weight_lastMC -37.903     46.769 181.567  -0.810    0.419
LineH:Temp30  -13.780     10.821 180.273  -1.273    0.205
LineL:Temp30   -5.314     11.039 182.789  -0.481    0.631
```

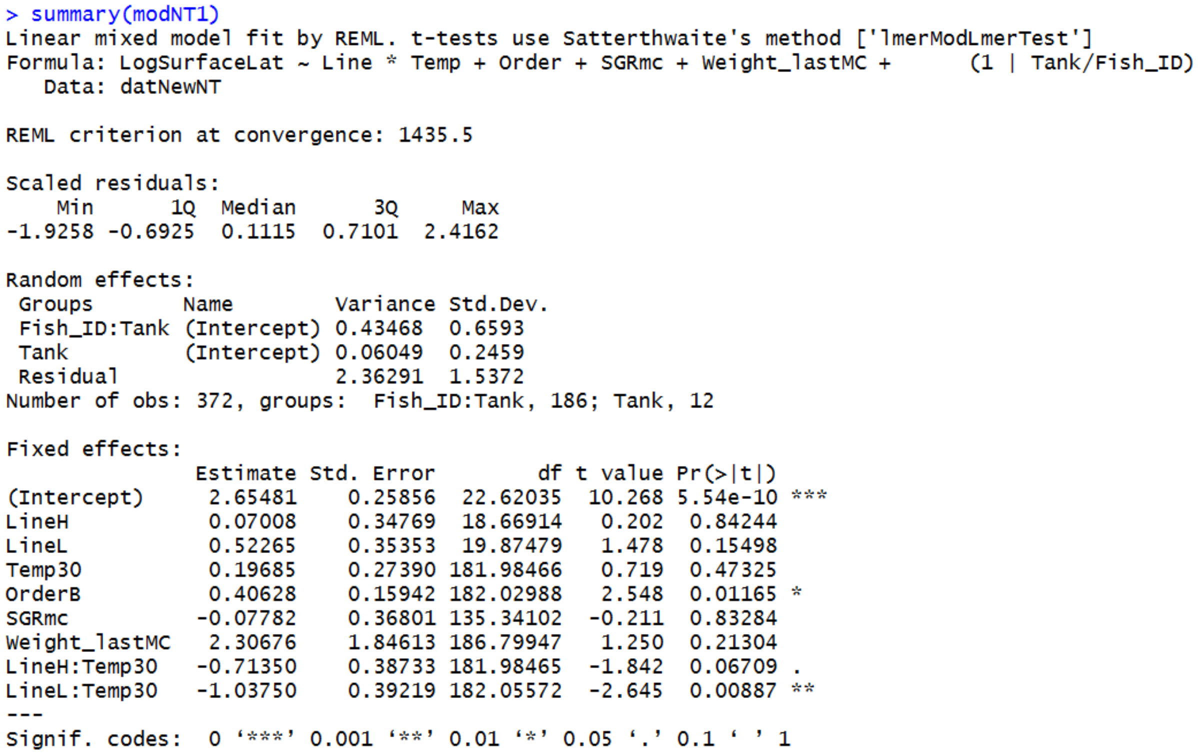


```
> anova(modA1)
Type III Analysis of Variance Table with Satterthwaite's method
               Sum Sq Mean Sq NumDF   DenDF F value Pr(>F)
Line           828.41  414.21     2   9.116  0.2266 0.8016
Temp          1838.43 1838.43     1 181.380  1.0057 0.3173
Order          456.09  456.09     1 181.427  0.2495 0.6180
SGRmc         2202.03 2202.03     1 170.721  1.2047 0.2739
Weight_lastMC 1200.59 1200.59     1 181.567  0.6568 0.4188
Line:Temp     3017.43 1508.71     2 181.437  0.8254 0.4397
```

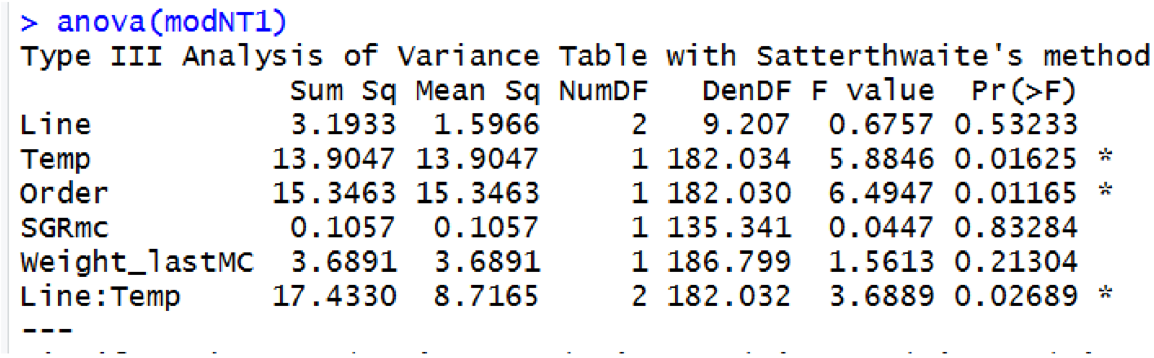
Latency summary and anova table

Novel tank treatment

modNT1 <- lmer(LogSurfaceLat ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID), na.action=na.omit, data = datNewNT, REML = T)



```
> summary(modNT1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: LogSurfaceLat ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNT

REML criterion at convergence: 1435.5

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-1.9258 -0.6925  0.1115  0.7101  2.4162 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 0.43468  0.6593  
 Tank         (Intercept) 0.06049  0.2459  
 Residual                 2.36291  1.5372  
Number of obs: 372, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
               Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)     2.65481    0.25856  22.62035  10.268 5.54e-10 ***
LineH           0.07008    0.34769  18.66914   0.202  0.84244    
LineL           0.52265    0.35353  19.87479   1.478  0.15498    
Temp30          0.19685    0.27390 181.98466   0.719  0.47325    
OrderB          0.40628    0.15942 182.02988   2.548  0.01165 *  
SGRmc          -0.07782    0.36801 135.34102  -0.211  0.83284    
Weight_lastMC   2.30676    1.84613 186.79947   1.250  0.21304    
LineH:Temp30   -0.71350    0.38733 181.98465  -1.842  0.06709 .  
LineL:Temp30   -1.03750    0.39219 182.05572  -2.645  0.00887 ** 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



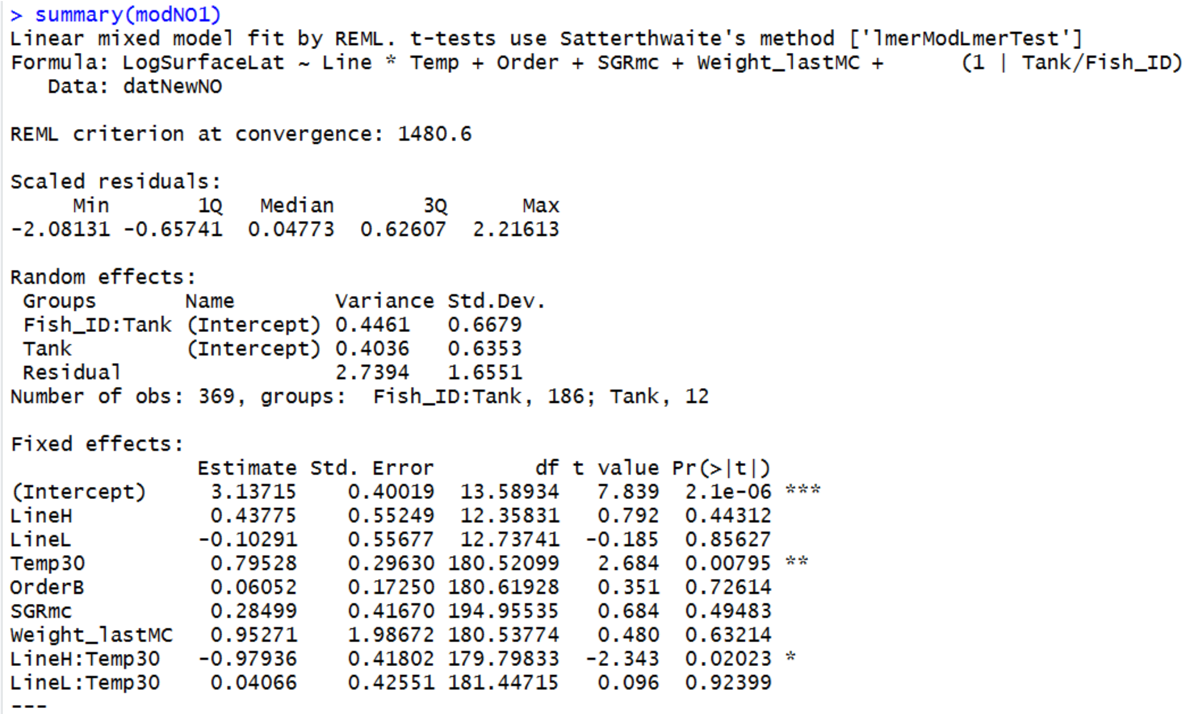
```
> anova(modNT1)
Type III Analysis of Variance Table with Satterthwaite's method
               Sum Sq Mean Sq NumDF   DenDF F value  Pr(>F)  
Line           3.1933  1.5966     2   9.207  0.6757 0.53233  
Temp          13.9047 13.9047     1 182.034  5.8846 0.01625 *
Order         15.3463 15.3463     1 182.030  6.4947 0.01165 *
SGRmc          0.1057  0.1057     1 135.341  0.0447 0.83284  
Weight_lastMC  3.6891  3.6891     1 186.799  1.5613 0.21304  
Line:Temp     17.4330  8.7165     2 182.032  3.6889 0.02689 *
---
```

Latency summary and anova table

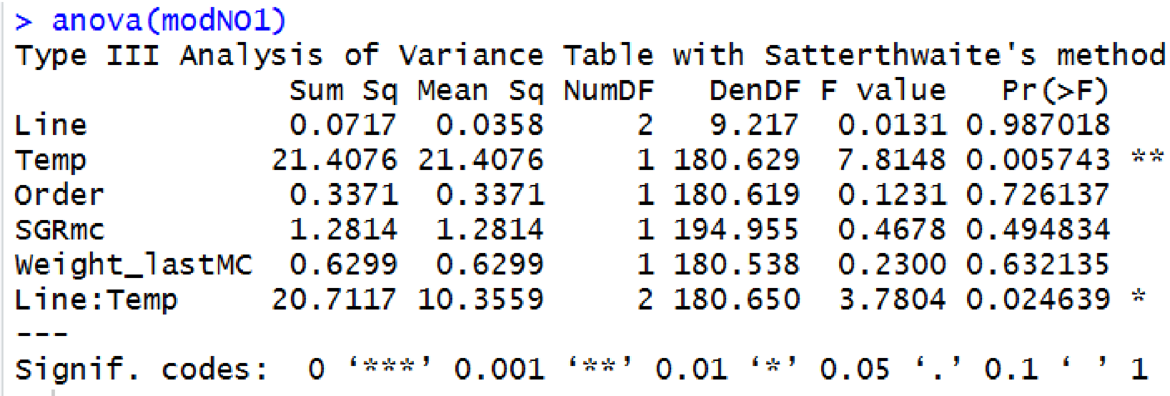
Novel object treatment

modNO1 <- lmer(LogSurfaceLat ~ Line*Temp + Order + SGRmc + Weight_lastMC + (1|Tank/Fish_ID),

na.action=na.omit, data = datNewNO, REML = T)



```
> summary(modNO1)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: LogSurfaceLat ~ Line * Temp + Order + SGRmc + Weight_lastMC +      (1 | Tank/Fish_ID)
   Data: datNewNO

REML criterion at convergence: 1480.6

Scaled residuals: 
     Min       1Q   Median       3Q      Max 
-2.08131 -0.65741  0.04773  0.62607  2.21613 

Random effects:
 Groups       Name        Variance Std.Dev.
 Fish_ID:Tank (Intercept) 0.4461   0.6679  
 Tank         (Intercept) 0.4036   0.6353  
 Residual                 2.7394   1.6551  
Number of obs: 369, groups:  Fish_ID:Tank, 186; Tank, 12

Fixed effects:
               Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)     3.13715    0.40019  13.58934   7.839  2.1e-06 ***
LineH           0.43775    0.55249  12.35831   0.792  0.44312    
LineL          -0.10291    0.55677  12.73741  -0.185  0.85627    
Temp30          0.79528    0.29630 180.52099   2.684  0.00795 ** 
OrderB          0.06052    0.17250 180.61928   0.351  0.72614    
SGRmc           0.28499    0.41670 194.95535   0.684  0.49483    
Weight_lastMC   0.95271    1.98672 180.53774   0.480  0.63214    
LineH:Temp30   -0.97936    0.41802 179.79833  -2.343  0.02023 *  
LineL:Temp30    0.04066    0.42551 181.44715   0.096  0.92399    
---
```



```
> anova(modNO1)
Type III Analysis of Variance Table with Satterthwaite's method
              Sum Sq Mean Sq NumDF   DenDF F value   Pr(>F)   
Line          0.0717  0.0358     2   9.217  0.0131 0.987018   
Temp         21.4076 21.4076     1 180.629  7.8148 0.005743 **
Order         0.3371  0.3371     1 180.619  0.1231 0.726137   
SGRmc         1.2814  1.2814     1 194.955  0.4678 0.494834   
Weight_lastMC  0.6299  0.6299     1 180.538  0.2300 0.632135   
Line:Temp    20.7117 10.3559     2 180.650  3.7804 0.024639 * 
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix B, Various visual representations

Table B1 Schematic overview of the colouration of the tagging. Left and right indicates sides of the fish. Fish 17 & 18 were extra.

Fish nr	Left	Right	Fish nr	Left	Right
1	🟥	🟥	10	🟨	🟧
2	🟥	🟧	11	🟨	🟨
3	🟥	🟨	12	🟨	🟩
4	🟥	🟩	13	🟩	🟥
5	🟧	🟥	14	🟩	🟧
6	🟧	🟧	15	🟩	🟨
7	🟧	🟨	16	🟩	🟩
8	🟧	🟩	17	🟪	🟦
9	🟨	🟥	18	🟦	🟪

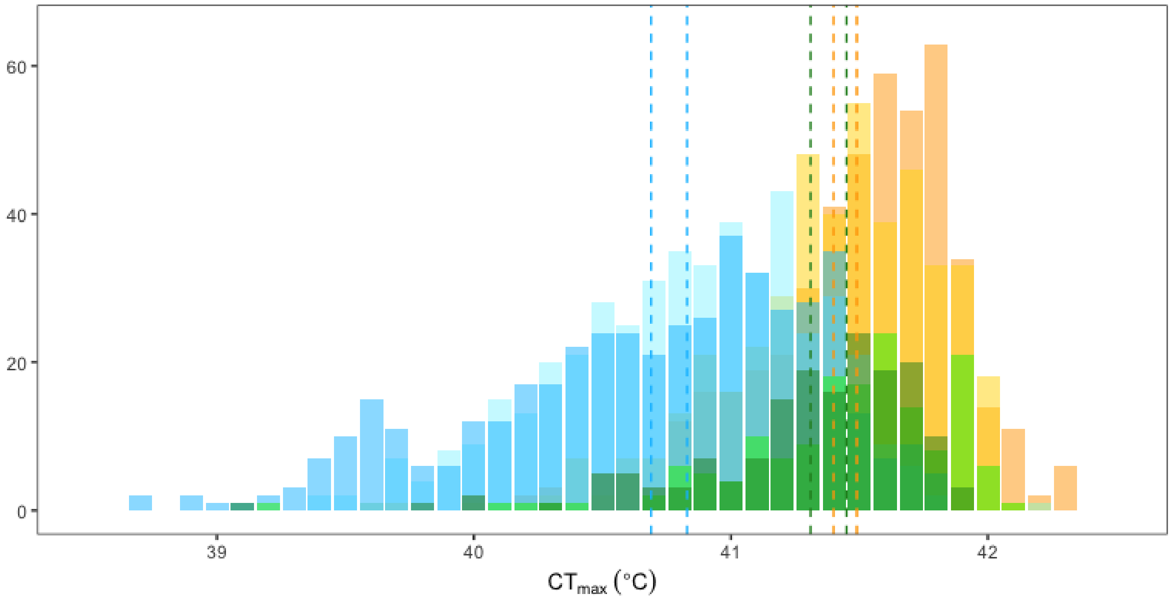


FIGURE B1: HISTOGRAM OF THE CTMAX RESULTS FOR THE 4TH GENERATION ZEBRAFISH IN THE JUTFELT ECOPHYSIOLOGY LAB, BY RACHAEL MORGAN. THE HISTOGRAM SHOWS A WIDER RANGE OF CTMAX FOR THE LOW LINE (BLUE) THAN THE HIGH LINE (YELLOW). RANDOM LINE ALSO HAS A WIDE S

Appendix C, Picture from the stalling.

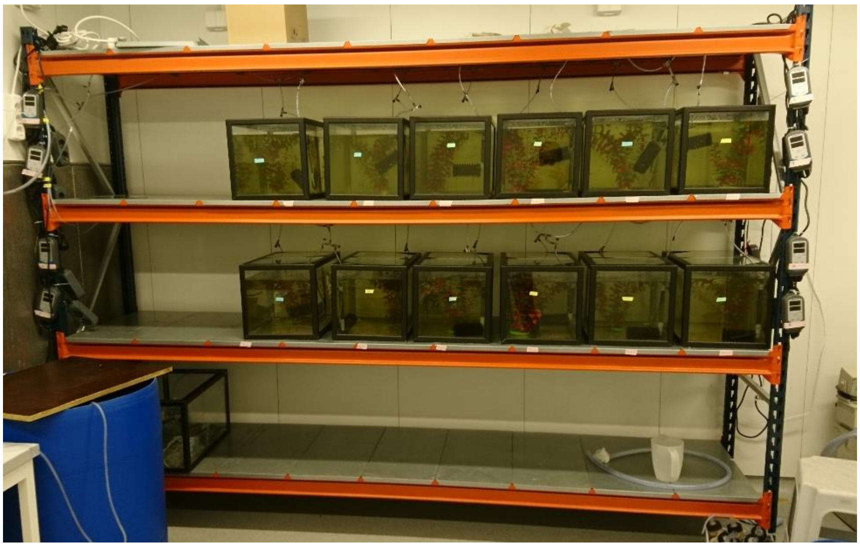


Figure C1: stalling arrangement for the zebrafish used in this experiment. each tank is labelled by line, replicate and tank number. The tanks were placed randomly

NTNU
Norwegian University of
Science and Technology

Jutfelt Fish
Ecophysiology lab