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Evolutionary change in metabolism of *Daphnia pulicaria* in response to the invasive predator *Bythotrephes longimanus*

Master's thesis in Biology
Supervisor: Sigurd Einum
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Abstract

Invasive predators have been identified as one of several threats to global biodiversity. This makes it necessary to understand their potential lethal and non-lethal impacts on native species, communities, and ecosystems. Interactions between invasive predators and native prey can lead prey to adapt as a part of an ‘evolutionary arms race’. Several studies suggest changes in behaviour, morphology, life history, and physiological characteristics of the prey as a result of direct selection imposed by the predator. However, much less emphasis has been directed towards indirect selective pressures induced by invasive predators, acting through a change in trophic control of the prey species (i.e., from bottom-up to top-down control). Metabolic rate is one of the candidate traits that can be hypothesised to evolve in response to such a change in trophic control, even in the absence of direct selection. This trait has been linked with essential physiological and life-history traits, such as growth, reproductive output, and survival, which makes it important to understand its evolutionary response to environmental change. In the current study I address this question by utilising an invasive event by the predatory zooplankton *Bythotrephes longimanus* in Lake Mendota, Wisconsin, US. This invasion dramatically impacted the prey species *Daphnia pulicaria*, causing a ~60% decline in their biomass. Resurrected clones from the pre-invasion period were obtained from resting eggs collected from sediment cores, and the metabolic rate of these was measured and compared with that of contemporary clones. Based on previous studies, I hypothesised the evolution of an increased metabolic rate in response to the predator invasion. However, the results showed a lower predicted metabolic rate for post-invasive clones than pre-invasive clones across the whole range of body lengths. One potential explanation for this observation is that the prey species remains bottom-up controlled due to a behavioural shift, whereby they increase their use of deeper, cooler, and less productive habitats. Thus, although this is one of the first studies to demonstrate evolutionary changes in metabolic rates in response to an invasive predator, further work is required to fully understand the evolution of this trait in response to ecological change.

Keywords- Invasive species, metabolic rate, evolutionary response, vertical migration, anti-predatory behaviour

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Table of content

Abstract.....	1
Acknowledgement.....	2
Introduction.....	4
Materials and methods.....	7-8
<i>Study species.....</i>	<i>7</i>
<i>Metabolic rate measurements.....</i>	<i>7</i>
<i>Statistics.....</i>	<i>8</i>
Results.....	9
Discussion.....	11
References.....	14
Supplementary materials.....	19

Introduction

Invasive predators are identified as one of several threats to global biodiversity. This makes it necessary to understand their potential lethal and non-lethal impacts on native species, communities, and ecosystems (Lima, 1998; Pangle & Peacor, 2006; Gillis & Walsh, 2017). Invasion by a non-native predator species may lead to a decreased population abundance or complete extinction of species in lower trophic levels. Furthermore, the predator-prey interactions can force the prey to adapt to reduce their loss of fitness (Gillooly et al., 2001; Barry & Sval, 2013; Krams et al., 2013) as a part of an 'evolutionary arms race' (Walsh et al., 2016). Some of the reported evolutionary responses in prey species include the changes in patterns of movement (Dodson & Havel, 1988; Bourdeau et al., 2013), changes in body size and reproductive effort (Fisk et al., 2007; Gillis & Walsh, 2017), increased ability to escape (O'Steen et al., 2002) or changes in feeding preferences (Singer et al., 1993). Most of these studies have focused on phenotypic traits in prey that are presumed to be under direct selection imposed by predation pressure. However, much less emphasis has been directed towards the indirect selective pressures induced by invasive predators, acting through changes to the direction of trophic control in a given ecosystem. Species in a certain trophic level can be regulated by either resource availability (bottom-up control) or by predation in a higher trophic level (top-down control) (Power, 1992). Invasive predator species, specifically those that themselves experience little predatory pressure in the new environment, have the potential to change the structure of a community through direct or indirect impacts on one or several species (Mooney & Cleland, 2001; White et al., 2006; Doody et al., 2015; Feit et al., 2018; Feit et al., 2020). These impacts become more influential if the affected prey species is a keystone species (Feit et al., 2018), and they even include the potential for complete reversal of the direction of trophic control (Walsh et al., 2017). However, it remains largely unknown how such changes in ecosystem structure might indirectly influence phenotypic optimality of life history and physiology, potentially adding another dimension to the evolutionary responses caused by invasive predators.

On an individual level, a change in the trophic control from bottom-up to top-down as a result of invasive predators entails a shift from being energetically challenged to having food in abundance. Candidate traits that can be hypothesised to evolve in response to a change in trophic control, even in the absence of direct selection, are therefore likely in some way to involve the conversion of energy. Metabolic rate (MR) may be one such trait. Metabolism is the set of enzymatic reactions that produce energy in a living being and thus has been connected to the rate of all biological activities (Brown et al., 2004). MR has been linked with essential

physiological and life-history traits, such as growth, reproductive output, and survival (Pettersen et al., 2018), is relatively easy to quantify and is present in all living organisms. This makes it a trait of high feasibility, generality, and importance in regard to the question of evolutionary responses to trophic control. Furthermore, the broad-sense evolvability in MR is similar in magnitude to other physiological and life-history traits, pointing towards potential for its evolution (Hansen et al., 2011; Einum et al., 2019). However, the potential for MR to evolve in response to the type of trophic control experienced by a given organism has not been investigated, although some studies have pointed towards the selection for low MR in low food conditions, which can be considered to resemble conditions in an ecosystem having bottom-up trophic control (Burton et al., 2011, Einum, 2014). For example, a study comparing guppy populations experiencing different predatory pressures supports this view, showing a higher MR in guppies originating from high predation environment (Auer et al., 2018).

Daphnia has been used as a model organism for evolutionary research in freshwater ecosystems thanks to its substantially investigated life cycle and easy rearing in laboratory conditions (Lampert, 2006; Miner et al., 2012). *Daphnia*'s ecology is well studied in terms of being a key link in freshwater food webs as a food source for fish and planktivorous organisms, influencing lower trophic levels by maintaining predatory pressure on phytoplankton, and recycling dissolved nutrition in plankton systems. This makes it an ideal candidate for ecological studies involving changes in trophic control and related consequences (Strecker & Arnott, 2008; Stolleweck, 2010). The dormant resting eggs (ephippia) of *Daphnia* are used in so-called 'resurrection ecology' research. Since the ephippia sink to the bottom of the water body and remain viable for years thereafter, they provide a temporal record of populations from which it is possible to observe the evolution of various physiological, behavioural and life-history traits in genotypes from different time periods (Frisch et al., 2014; Landy et al., 2020). *Daphnia* has been reported to show adaptive plasticity in MR in the presence of predators (Handelsman et al., 2013), but currently, there is a lack of studies regarding the potential for evolutionary responses in MR.

In the current study, I address the question of an evolutionary response in MR to a change in trophic control by studying a population of *Daphnia pulicaria* from Lake Mendota, Wisconsin, USA. This lake has experienced the introduction of zooplanktivorous predator, *Bythotrephes longimanus*, native to Northern Europe (Strecker et al., 2006; Hasnain & Arnott, 2019). This predator was first observed in 2009 in various lakes in Wisconsin (Walsh et al., 2016). Studies suggest its presence in lakes before the year 2009, as evident by the recovery of spines in the lake sediments. Their main ecological impact happened after 2009, however, as supported by

a sharp and significant decrease in *Daphnia pulex* population in Lake Mendota (Walsh et al., 2016; Landy et al., 2020). The invasion by *Bythotrephes* caused a ~60% decline in the biomass of *Daphnia pulex*, followed by one-metre decrease in Secchi depth, indicating a substantial increase in phytoplankton biomass (Walsh et al., 2016). This sudden increase in food availability for the remaining *Daphnia* population might have led it to adapt an increased MR. Thus, I hypothesise that the evolution in MR of an organism should be positively related to the increase in top-down regulation. I tested my hypothesis by measuring and comparing the MR of *Daphnia pulex* clones from two time periods – before and after *Bythotrephes longimanus* invasion, in Lake Mendota reared in a common garden experiment. Multiple linear mixed models were fitted to test the data since both fixed and random effects are expected to affect the MR in my experiment.

Materials and Methods

Study Species

Duplicate sediment cores were collected in February 2018 from Lake Mendota in Wisconsin using 1.5 m Griffith sediment corer with Livingstone drive rods. The cores were then analysed using ^{210}Pb dating and loss-on-ignition assays to identify the demarcating depth for the pre- and post-invasion period. A depth of 2 cm was found to be the transition point between pre- and post-invasion period in Lake Mendota. If a trait is estimated using individuals from the same clone line, it can give an approximately true independent estimate of the mean clonal value (Einum et al., 2019). Thus, multiple clones were hatched from resting eggs found in the sediment cores and grouped into pre- and post-invasion clones. In December 2019, live individuals of the different clones were transported to NTNU.

The selected clones were cultured and maintained at a population density of 10-15 individuals in 80 ml plastic jars filled with 50 ml selenium-modified ADaM (Kluttgen et al., 1994, SeO_2 concentration reduced by 50%) at a temperature of 17°C and 16-hour photoperiod in Memmert Peltier incubators (Memmert GmbH). The medium was replaced every two weeks, at which point the position of jars within the cabinet was changed randomly. Each jar received Shellfish Diet 1800 (Reed Mariculture Inc, Campbell, CA, USA) three times per week throughout the experimental duration at a cell concentration of 4×10^5 cells/ml.

Metabolic Rate Measurement

Direct measurement of metabolic rate is difficult and, therefore, the rate of oxygen consumption, i.e., respiration rate, is usually used as a substitute measurement to estimate the MR (Fuhrman et al., 1961; Barry & Syal, 2013). The MR was measured as oxygen consumption at 17° C using a 24-channel fluorescence-based respirometry system (SDR SensorDish Reader, PreSens, Germany) placed in an incubator (Memmert Peltier-cooled incubator IPP, Germany). The oxygen consumption rate was measured using a glass microplate (24-wells with a capacity of 200- μl) equipped with planar oxygen sensor. The wells were pre-filled with air saturated ADaM before the transfer and sealed with an adhesive PCR film (Thermo Scientific, Waltham, USA). The effects of changing temperature on metabolic rate were minimised using a water bath at 17° C during the transfer of individuals into the microplate and the sealing process. Oxygen concentration readings in the microplate were taken every 3 minutes in darkness for 1.5 to 2 hours using SDR version 38 software (PreSens, Germany). After the oxygen consumption measurement, all individuals were photographed under a stereomicroscope (Leica Microsystems GmbH) and measured digitally for body length (millimetres); from the tip of head crest to the point of tail spine attachment to the carapace, using IMAGEJ version 1.49v software (National Institutes of

Health, Bethesda, MD, USA). The MR was calculated by regressing oxygen consumption on time following the Yashchenko et al. (2016) method.

The experiments were conducted using only female individuals. Twenty-two different clones were used, with eleven from the pre-invasion and eleven from the post-invasion period. Prior to an experimental run, two individuals from each clone were taken and kept in isolation in 50ml ADaM for 48-54 hrs. They were fed two times before measurements. A total of six runs with twenty-two individuals (one in each well) per run were conducted, resulting in 418 measurements. However, fifty-six of these were excluded from statistical analyses due to individuals being injured during the process or air bubbles being observed within the wells following measurements. In each run, two 'blank' wells containing only ADaM were used as controls to estimate the level of microbial respiration, and the mean of this was subtracted from the observed oxygen consumption.

Statistics

I used linear mixed effect modelling to model MR as a response to body length and clone type (pre-and post-invasion) as fixed effects and with run and clone line as random effects. Four different models with different fixed terms but the same random effects were fitted. The first model included the additive effect of body length and clone type. The second model contained both body length and clone type as well as their interaction. The third and fourth models used only body length and clone type as fixed effects, respectively.

Models were compared using Akaike Information Criterion (AIC) after fitting with maximum likelihood, while parameter estimates of the two best models were obtained by refitting the models with restricted maximum likelihood. The two models with the highest support were inspected for linearity, homoscedasticity, and normal distribution of residuals (see Supplementary Materials). All statistical analyses were done using R v. 4.0.2 (RCore Team, 2020), and linear mixed-effect models were fitted using the *lmer* function in the *lme4* package (Bates et al., 2015).

Results

When comparing the AIC values for the different models, the two models (model 1 and model 2) that contained effects of both body length and clone type received considerably stronger support than those containing only one of these terms (Table 1). Support for the two top models, with one only containing additive effects of body length and clone type, and one including an interaction between these, was similar in magnitude. For the model with only additive effects, MR increased with body length, and post-invasion clones had a lower MR than pre-invasion clones (Fig. 1a). However, the interaction term was relatively weak, with the 95% CI including zero (Table 2), and the predicted MR for post-invasion clones was lower than that for the pre-invasion clones across the whole range of body lengths (Fig. 1b).

For the model with only additive effects, the predicted MR of an individual of mean body length was 2.21 mm and 2.19 mm for the pre- and post-invasive clone type, respectively. Thus, the post invasive clones had a reduction of 7.7% in MR compared to the pre-invasive clones.

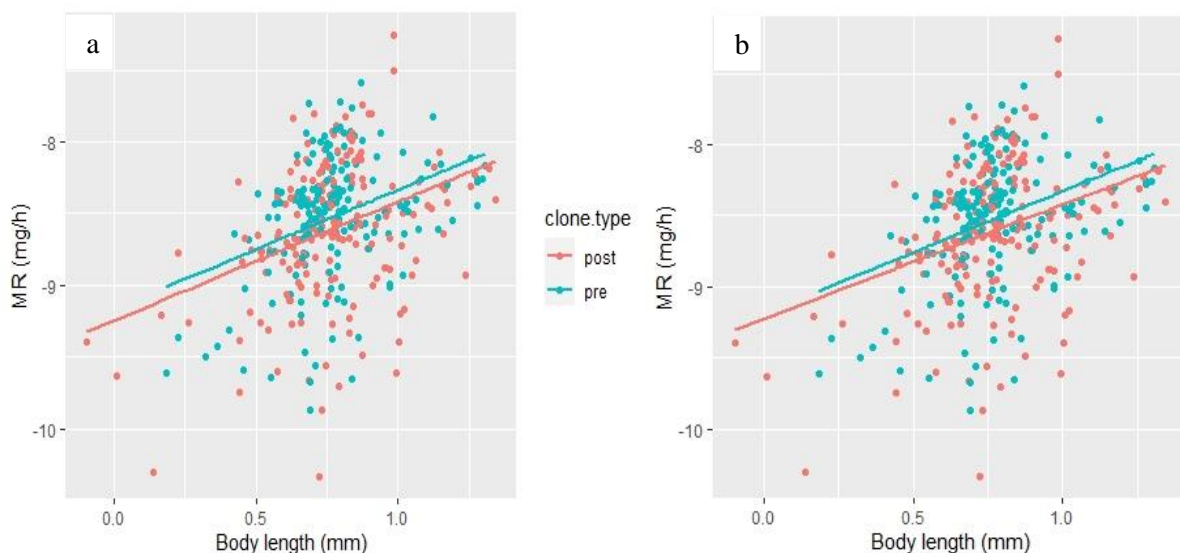


Fig 1. Relationship between metabolic rate (MR) and body length for pre-invasion and post-invasion clones of *D. pulicaria* from Lake Mendota, U.S. measured with (a) additive effect of body length and clone type (b) interaction between body length and clone type

Table 1: Alternative models testing the relationship between metabolic rate (MR) in *D. pulicaria* in relation to body length and clone types (pre and post- invasive). The models were fitted with Maximum likelihood (ML)

Models	Fixed variables	K	AIC _C	ΔAIC _C	w _i	acc w _i	R ²
1	Body length + Clone type	6	303.23	0	0.48	0.48	0.698
2	Body length * Clone type	7	303.48	0.24	0.43	0.9	0.703
3	Body length	5	306.58	3.35	0.09	1.0	0.693
4	Clone type	5	451.11	147.88	3.73x10 ⁻³³	1.0	0.186

K: number of parameters (K-2) in model. **ΔAIC_C:** difference in AIC_C values between the given model and best-fitting model among the candidates. **w_i:** AIC_C weight or likelihood of the given model being the best model among the candidates. **acc w_i:** sum of AIC_C weights. **R²:** conditional r-squared for given model, estimated using the MuMIn package (Bartoń, 2020) in R.

Table 2: Summary of the two best models estimating MR in relation to body length and clone types (pre and post-invasion). Model 1 used the additive effect of body length and clone type as fixed effects. Model 2 used the interaction of body length and clone type as fixed effects. The models were fitted with Restricted Maximum Likelihood.

	Model 2		Model 1	
	Log (MR)		Log (MR)	
<i>Predictors</i>	<i>Estimates</i>	<i>95% CI</i>	<i>Estimates</i>	<i>95% CI</i>
(Intercept)	-9.95	-10.22 – -9.69	-10.02	-10.27 – -9.78
Body length [log]	1.75	1.47 – 2.02	1.83	1.60 – 2.07
Clone type [pre-invasive]	-0.11	-0.39 – 0.18	0.08	0.01 – 0.15
Body length [log] * Clone type [pre-invasive]	0.25	-0.11 – 0.61		
Random Effects				
σ ² (Variance)	0.11		0.11	
τ ₀₀ (Random intercept variance)	0.00 Clones		0.00 Clones	
	0.13 Rununique		0.12 Rununique	
N	19 Rununique		19 Rununique	
	21 Clones		21 Clones	
Observations	356		356	

The summaries were created using sjplot package (Lüdecke, 2021) in R.

Discussion

This study aimed to test if the invasion of a non-native predator can cause an evolutionary change in the MR of its prey species. I used *Daphnia pulicaria* clones from Lake Mendota, US, as the study prey species since this lake has been documented to have an established population of the non-native predator *Bythotrephes longimanus* that have caused large cascading effects on phytoplankton abundance (Walsh et al., 2016). This invasion has shifted the trophic control from bottom-up to top-down, which might result in abundant food availability for the surviving *Daphnia*. Since low food conditions have been connected with selection for lower MR (Burton et al., 2011; Einum, 2014), high food conditions should lead to selection for high MR.

The results in my study suggest a lower metabolic rate among post-invasive clones, thus contradicting my hypothesis of an increase in metabolic rate as an evolutionary response to predator invasion. A previous study on metabolic rate evolution in guppies has suggested a consistent increase in the MR in high predation environments (Auer et al., 2018). However, this does not necessarily contradict the results of my study. The guppies and *Daphnia* live in different environments, lotic and lentic, respectively, and face different predators. It is unclear whether the predators preying on guppies and *Daphnia* affect their respective ecosystem with similar intensity. It is therefore not surprising that the prey species could develop vastly different strategies to avoid predation. In guppies, a high pace life history, i.e. low age at maturation, faster reproduction rate and shorter life span, was connected with higher metabolic rate (Auer et al., 2018). The data from *Daphnia* collected in common garden experiment show higher growth rate and larger size at maturation in populations from lakes invaded with *Bythotrephes*; however, they do not show a significant difference in the age at maturation (Walsh et al., 2016). This might imply that *Daphnia*, unlike the guppies, do not increase their life history pace in the presence of a predator but instead adapt the efficiency of their metabolism to lower rates of intraspecific competition. Ullern (2020) reported that post invasive clones from the same lake as used in the current study have a higher growth rate at low population density, but that an increase in population density more negatively impacted them compared to pre-invasive clones. It could be the case that high rates of intraspecific competition necessitate higher metabolic rate to facilitate more efficient exploitation of a limited food supply. However, when food is abundant, for example, following the invasion of *Bythotrephes*, it might be the case that metabolic rate can actually be optimised to enable more efficient growth and reproduction. This could coincide with the pattern simulated in Einum

(2014), which indicates that optimal resting MR peaks at intermediate food availability but decreases with higher food availability.

Another possible explanation for the decline in MR observed in my study might be the reported preference of *Bythotrephes* for prey near-surface leading *Daphnia* to migrate vertically to darker, cooler and less productive habitat following the *Bythotrephes* invasion (Pangle et al., 2006; Strecker & Arnott, 2008). Vertical migration in response to predator presence in *Daphnia* has a genetic component that can lead a few individuals to develop an ability for vertical migration to deeper habitat (Rozenberg et al., 2015; Hasnain & Arnott, 2019). The lower food and oxygen availability in deeper habitat caused by less light penetration, less oxygen mixing, and low temperature can force the prey to keep a lower metabolic rate (Dawidowicz et al., 2013; Hoefnagel et al., 2018). In such a case, the population would still experience the condition that resembles the state of bottom-up trophic control.

Changes in the anti-predatory behaviour in presence of an invasive predator could also explain the results of my study. A prey will possibly be less active if threatened by constant exposure with any predator, to increase the survival probability by decreasing the encounter rate. Such conditions may lead *Daphnia* to reduce activity and consequently to have lower energy intake, reduced growth, and lower metabolic rate. Under this scenario, any indirect selective effect of the predator acting through a change in trophic control would be counteracted by the direct selective effect against high levels of activity.

The absence of predators for *Bythotrephes* and its established population in Lake Mendota points towards a permanent predator threat for *Daphnia*. The large size and feeding appendages of *Bythotrephes* may contribute to posing a greater predatory pressure on *Daphnia* compared to their other cladoceran predators (Foster, 2009; Bourdeau et al., 2013). The direct presence of a predator may lead the prey to sharply increase its MR in a plastic flight response to increase its survival chances in the short term. However, if the stimulus for flight response would be permanently present, the energy cost of maintaining such a state will be highly costly to the prey. If the predator presence is a predicted and permanent part of prey's life cycle, it can be expected to adapt permanently instead of choosing a plastic change to decrease the energy cost.

The results might also have been different if kairomones or live predators were used in my study in the developmental stage of *Daphnia*. The current environment of a species may play a significant role in shaping its direction of response. The species may respond through phenotypic plasticity, direct evolution in the phenotype, or the evolution of the range of phenotypic plasticity. Phenotypic plasticity is the ability of a genotype to express different

phenotypes as a response to environmental variation. The evolution of phenotypic plasticity would then be a change in the range of phenotypes possibly expressed by one genotype. Landy (2020) study, however, might imply that the plasticity of MR is not evolving because the plasticity of life history and behavioural traits associated with MR is not evolving in the presence of *Bythotrephes* in Lake Mendota.

The extent and direction of change in metabolic rate as the response to predators seem to be species-specific. A prey species usually faces conflict between meeting energy demands and predator avoidance (Brown et al., 2006; Barry & Syal; 2013), and the strategy to deal with predator-induced mortality can be different for different species, as suggested by several studies (Steiner & Buskirk, 2009; Barry & Syal, 2013; Antol et al., 2018). As an example of plastic change of MR, Barry & Syal (2013) reported a decrease in respiration rate in tadpoles as a response to predator dragonfly larvae by few adjustments in their physiology to partially balance the cost of lower energy intake considering the predator avoidance. Another report on tadpoles demonstrated higher oxygen consumption during short-term exposure to a predator with a decline in response after long-term exposure, indicating the highly plastic nature of the physiological responses to predation risk and its relation to the time scale of exposure (Steiner & Buskirk, 2009). The zebra mussels have been observed to initially decrease the oxygen consumption rate and then steadily increase it over time in the presence of predator cues (Antol et al., 2018). The zebra mussels have also been reported to respond differently to distinctive predators in terms of rapid or slow changes in metabolic rate (Antol et al., 2018).

Further experimental studies can be conducted with samples from different lakes with the same and different invasive predators to generalise the current study's outcomes. Using more lakes and a higher sample size can be helpful to put forward a stronger argument against the possibility of confounding effects due to temperature change, nutrition load, or any other undetected factor. The invasive predator and prey from different trophic levels should also be tested to see if the response changes with a change in size and type of predation pressure.

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Supplementary material

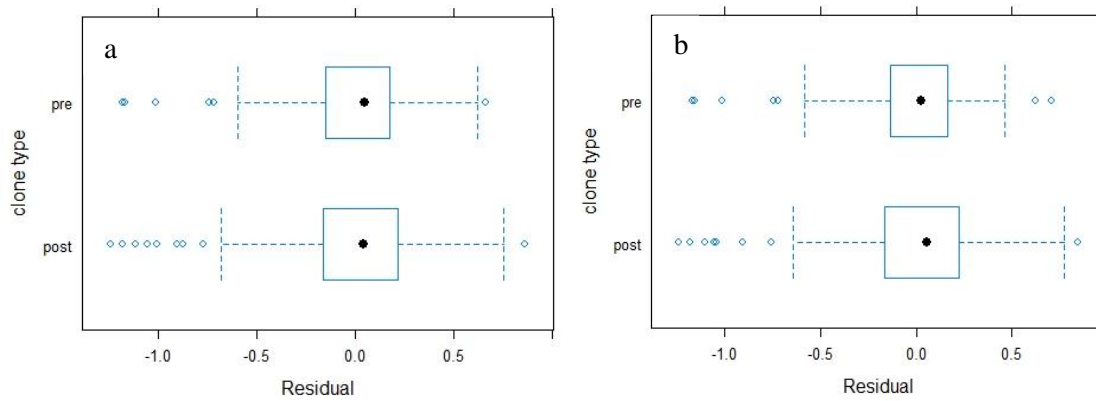


Fig 2 – Residuals for clone types of (a) model with an additive effect of body length and clone type (b) model with interaction between body length and clone type

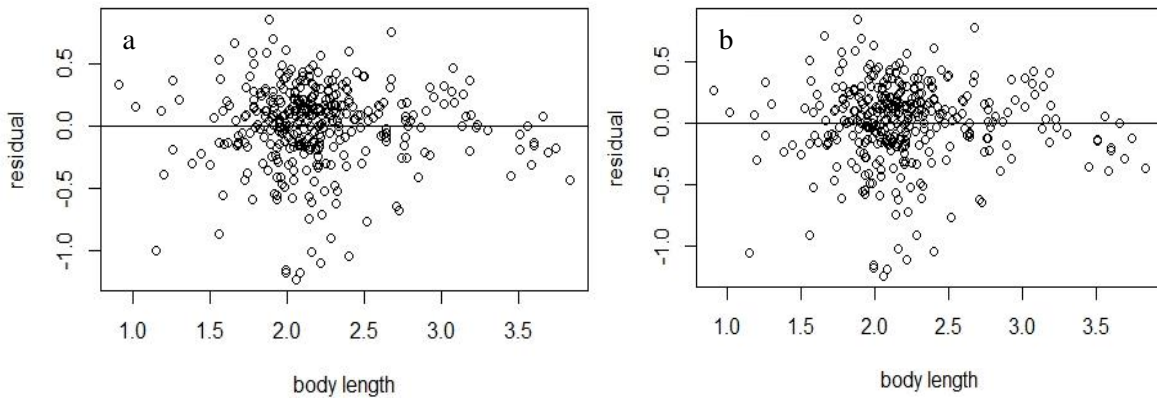


Fig 3– Residuals for body length of (a) model with an additive effect of body length and clone type (b) model with interaction between body length and clone type

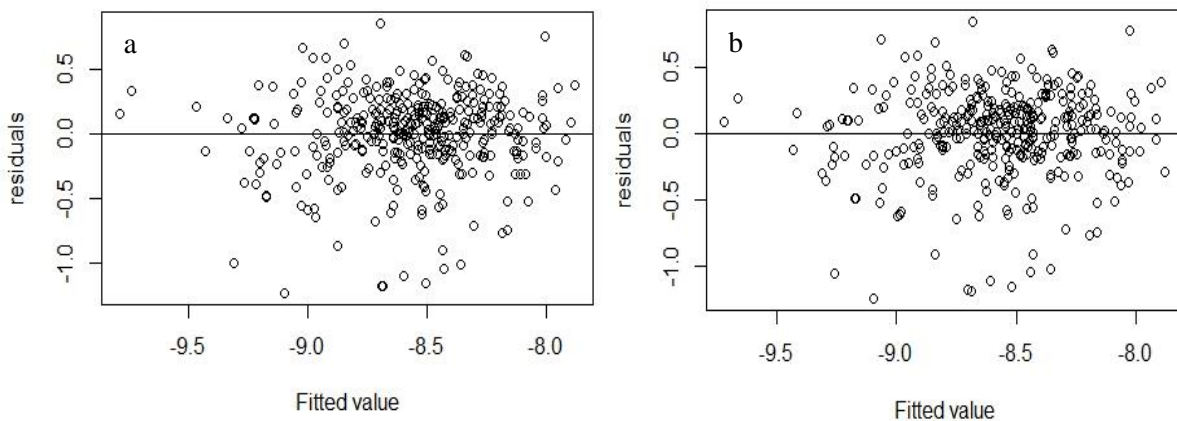


Fig 4– Fitted vs residual plot of (a) model with an additive effect of body length and clone type (b) model with interaction between body length and clone type

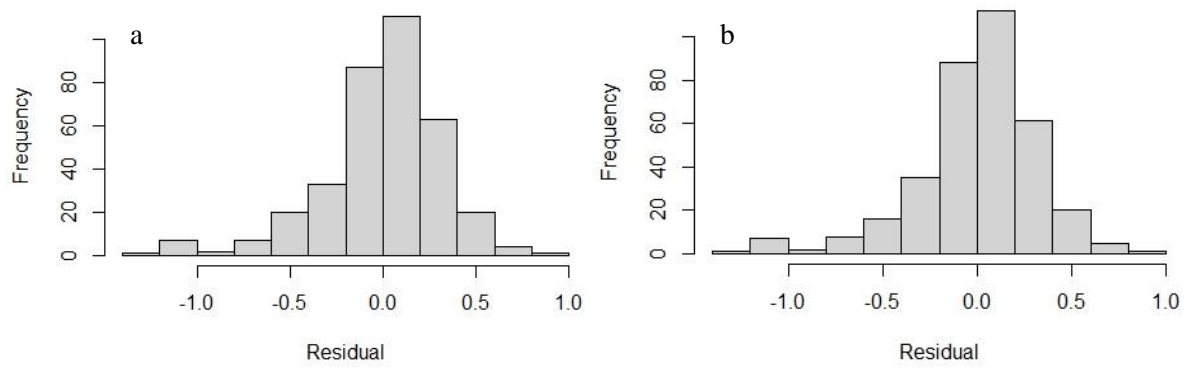


Fig 5 – Histograms for residuals of (a) model with an additive effect of body length and clone type (b) model with interaction between body length and clone type

