# ADAPTIVE AND NON-ADAPTIVE PLASTICITY AND FINE-SCALE GENETIC VARIATION IN LIFE-HISTORY REACTION NORMS IN ATLANTIC COD (*GADUS MORHUA*)

by

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Signature of Author

For my parents, who sparked my interest in the natural world.

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# Abstract

The persistence of a species in the face of environmental change is a function of the extent to which populations respond differently to changes in their environment and the spatial correspondence between the scale of disturbance and the scale of adaptation. The pattern by which a population, or genotype, expresses a range of phenotypes across an environmental gradient is called a norm of reaction. The level of phenotypic plasticity displayed within a population (i.e. the slope of the reaction norm) reflects the short-term response of a population to environmental change while variation in reaction norm slopes among populations reflects the spatial scale of variation in these responses. Using a reaction norm framework, I examined the spatial scale of genetic variation in plasticity for life-history traits in Atlantic cod (*Gadus morhua*), a marine fish of global biological and socioeconomic importance. Through common-garden experiments, I found evidence of both adaptive and non-adaptive plasticity for larval growth rate and survival in two cod populations that experience contrasting thermal environments in nature. A comparison of these reaction norms with those of four cod populations studied previously revealed significant genetic divergence in adaptive traits at a smaller spatial scale than has previously been shown for a marine fish with no apparent physical barriers to gene flow (<250 km). This fine-scale genetic structure is likely the result of populations being locally adapted to seasonal changes in temperature during the larval stage caused by differences in spawning times and may be maintained by behavioural barriers to gene flow. Implications of variation in life-history trait plasticity to fisheries management in the face of predicted changes in climate are discussed.

# List Of Abbreviations And Symbols Used

NAFO – Northwest Atlantic Fisheries Organization

SD – standard deviation

SE – standard error

SNP – single nucleotide polymorphism

TMS – tricaine methanesulfonate

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# Chapter 1: Introduction

In the face of environmental disturbance, the future of a species depends on the extent to which populations respond differently to changes in their environment and the spatial correspondence between the scale of disturbance and the scale of adaptation. In marine species, there is increasing evidence that the spatial scale of adaptation is smaller than previously understood (e.g. Ruzzante et al. 2006; Hutchings et al. 2007; Olsen et al. 2008; Gaggiotti et al. 2009). Traditionally, marine species were assumed to be genetically homogeneous due to a lack of apparent geographic or physical barriers to gene flow in the ocean (Hilbish 1996) coupled with the high dispersal capability of many species (Levin 2006). Support for this assumption comes from neutral genetic markers (e.g. microsatellites) that are routinely used in fisheries management to delineate population structure (Ward 2000; Kapuscinski & Miller 2007). However, neutral markers have limited ability to resolve patterns of genetic variation in species that experience relatively high levels of gene flow (Waples 1998) and are unable to detect structure that is the result of selection.

These limitations can be detrimental from a conservation perspective. For example, management of a stock as a whole could lead to the extinction of unique, locally adapted subpopulations and a consequent decrease in overall productivity (Frank & Brickman 2000). Population divergence can also occur more quickly in adaptive traits compared to neutral markers, particularly if selection pressures are strong (Hard 1995; Conover 1998; Conover et al. 2006), which can make them extremely useful for delineating populations. Genetic variation for traits related to fitness is also more likely to be relevant to population productivity and species persistence than neutral markers (Hard 1995; Conover and Munch 2002; Olsen et al. 2008). Therefore examining the spatial distribution of variation in adaptive traits can be helpful in understanding how populations and species are likely to be affected by directional changes in the environment, such as those hypothesized to be associated with climate change.

The pattern by which a genotype, or group of related genotypes, expresses a range of phenotypes across an environmental gradient is called a norm of reaction (Woltereck 1909; Schmalhausen 1949). A plastic, non-evolutionary response to environmental change is expected to shift the phenotype along the norm of reaction while a genetic, evolutionary response is expected to shift the reaction norm itself (Ernande et al. 2004). The slope of a reaction norm represents the amount of phenotypic plasticity displayed within a population and the short-term response to environmental change, which can be adaptive, maladaptive, or neutral with regard to individual fitness (Ghalambor et al. 2007). The spatial scale of variation in these responses is reflected by variation in reaction norm slopes among populations and reflects the long-term potential of a species to adapt to environmental change.

Using a reaction norm framework, I examined the spatial scale of genetic variation in life-history trait plasticity in a marine species of global biological and socioeconomic importance. Atlantic cod (*Gadus morhua*; hereafter, cod) is a demersal marine fish inhabiting coastal waters throughout the North Atlantic. The collapse of Canadian cod stocks in the early 1990s was biologically, socially, and economically devastating (Templeman 2010; Hutchings and Rangeley 2011). Despite a moratorium on fishing since 1992, most stocks have shown little or no recovery (Hutchings 2000; Hutchings and Rangeley 2011). However, variable rates of recovery among stocks underscore the need to understand the underlying genetic differences among stocks for traits that are likely to be influencing recovery.

Cod are subject to a variety of ecological conditions and selective pressures across their range that could potentially promote adaptive divergence among populations. Differences in ocean temperature and salinity regimes, duration of sea ice cover, fishing, and other predation pressures all have the potential to promote genetic differentiation in important life-history traits among groups. In addition, different groups of cod spawn at different times of year (e.g. Lett 1980; Brander & Hurley 1992; Myers et al. 1993) and some groups are highly migratory while others are not (e.g. Sinclair & Currie 1994; Ruzzante et al. 1998). Coupled with environmental variation, this behavioural variation

among spawning groups is expected to manifest in adaptive genetic differences among spawning groups at small spatial scales. There is evidence of divergence in life-history traits (Olsen et al. 2008) and sequence variation at the pantophysin locus (Pogson & Fevolden 2003) among neighbouring populations of coastal cod in Norway, attributed to the retention of offspring within fjord basins and natal homing of adults coupled with selection. Bradbury et al. (2010) showed variation in single nucleotide polymorphism (SNP) allele frequencies at temperature-associated genes at scales of 500-1000 km. Such genetic variation provides inferential evidence of genetic differences in functional responses to temperature.

Genetic variation in thermal responses can be assessed more directly through common-garden experiments in which the phenotypes of individuals from putatively different genetic groups are observed at a range of temperatures under controlled laboratory conditions (e.g. Conover & Present 1990; Schultz et al. 1996; Conover et al. 1997; Yamahira et al. 2007). By controlling for environmental influences, observed variation can be attributed to genetic differences between groups (assuming maternal effects are controlled to the greatest extent possible; Conover & Baumann 2009). Common-garden experiments in cod have revealed genetically variable thermal responses for body shape at both small (<100 km) and broad (>1000 km) spatial scales (Marcil 2004; Marcil et al. 2006a) and larval growth rate and survival at spatial scales of >600-800 km in the Northwest Atlantic (Hutchings et al. 2007).

Larval growth and survival are two life-history traits of paramount importance to the viability of cod populations. Faster growth during the larval and early juvenile stages is thought to be adaptive by shortening the length of these stages, during which the fish are most vulnerable (Anderson 1988; Steinarsson & Björnsson 1999) and mortality rates are very high (Houde & Zastrow 1993). Because the majority of mortality occurs during the larval stage, small differences in early life-history traits among populations can translate into large consequences for population productivity (Pörtner & Peck 2010). Reaction norms for survival can be particularly informative regarding potential adaptation due to the high association of survival with fitness (Hutchings 2011).

Both Marcil et al. (2006) and Hutchings et al. (2007) examined plasticity at a relatively high and narrow range of temperatures (7-11°C). Yet, not all populations are typically exposed to these temperatures during the larval stage (R. Oomen, unpublished data; see Appendix A, Figure 1 [A1] for details). It is therefore unlikely that these populations would have experienced the selective pressures necessary to shape an adaptive norm of reaction for these temperatures. In this case, the observed response may be the product of a genetic constraint (Angilletta et al. 2004) or neutral variation (Ghalambor et al. 2007) rather than adaptation. Examining population reaction norms outside the range of environments typically experienced in the wild can be useful for predicting the short-term response of a population to directional changes in their environment, but to examine the influence of previous environments on reaction norm evolution requires the study of the portion of the reaction norm that selection has had opportunity to act on.

This thesis expands on previous common-garden research conducted on cod by increasing the range of environments for which the reaction norm is examined and reducing the spatial scale at which population differences in plasticity for larval growth and survival are assessed. Chapter 1 serves to introduce both Chapter 2 and Chapter 3. Chapter 2 discusses common-garden experiments I conducted from 2011-2012 on two cod populations from different regions of the Northwest Atlantic that experience contrasting thermal environments in early life. The range of temperatures used in these experiments was extended beyond those previously examined for these traits. The primary objectives of this chapter were: 1) to improve our understanding of the range of thermal environments at which genetic divergence in plasticity exists for cod, and 2) to examine both the potentially adaptive and non-adaptive portions of reaction norms. In Chapter 3, the reaction norms constructed in Chapter 2 are compared with those reported by Hutchings et al. (2007) from 2002-2003. Spatial variation in reaction norms is assessed using all populations and temporal variation in reaction norms is assessed using a study population that is common to both sets of common-garden experiments. The objectives of this chapter are threefold: 1) to assess the manner in which reaction norms

based on common-garden experiments conducted at different times can be compared between populations, 2) to determine whether genetic variation in reaction norms for larval growth and survival exists between cod populations at a small spatial scale of ≈ 250 km, and 3) to investigate the role of spatial variation in thermal regimes in promoting adaptive divergence among local cod populations. In Chapter 4, implications of the findings of the present study are discussed.

Knowledge of the spatial scale of genetic variation for plasticity in life-history traits will contribute to a holistic assessment of population structure in the Northwest Atlantic and reflect population differences in short- and long-term responses to environmental change. These contributions are essential components for developing effective management strategies for Atlantic cod and may serve as a model for conserving other marine fishes with similar life-histories. From an evolutionary perspective, improved knowledge of the capacity for phenotypic change and how this capacity evolves will provide a fundamentally important empirical basis for predicting how natural and anthropogenic environmental variability will affect animal populations.

# Chapter 2: Adaptive And Non-adaptive Plasticity

## 2.1 Methods

### *2.1.1 Study Populations*

Two common-garden experiments were conducted from 2011-2012 on two putative Atlantic cod populations (Figure 1): 1) Southern Gulf of St. Lawrence (Northwest Atlantic Fisheries Organization [NAFO] division 4T, 47°N, 61°W), and 2) Southwestern Scotian Shelf near Sambro, Nova Scotia (NAFO division 4X, 44°25'N, 63°30'W). Cod from these areas will be referred to throughout this chapter as 4T and 4X-Sambro, respectively.

Southern Gulf of St. Lawrence cod are highly migratory, overwintering in Sydney Bight (NAFO division 4Vn) from November to April (Sinclair & Currie 1994; Comeau et al. 2002) and returning to the Southern Gulf of St. Lawrence (NAFO division 4T) for the summer months (May-October). Although spawning occurs in the Southern Gulf of St. Lawrence from April to September (ICES 1994), the peak spawning season occurs in May and June (Lett 1980). 4T larvae experience relatively warm and highly variable temperatures, increasing from 4±2(SD)°C in May to 10±4°C in August (R. Oomen, unpublished data; see Appendix A, Figure 1 [A1] for details; note the relatively large standard deviations representative of greater thermal variability).

Little is known about the group of cod that spawn off the coast of Sambro, Nova Scotia (NAFO division 4X), but if they are like other spawning components in the 4X division, they may experience limited migration (Ruzzante et al. 1998). Sambro cod have a unique fall spawning season with a peak spawning period from November to December (Brander & Hurley 1992; Hutchings et al. 1999). 4X-Sambro larvae experience relatively cold and less variable temperatures, decreasing from 9±2°C in November to 2±2°C in February (Figure A1; note the relatively small standard deviations).

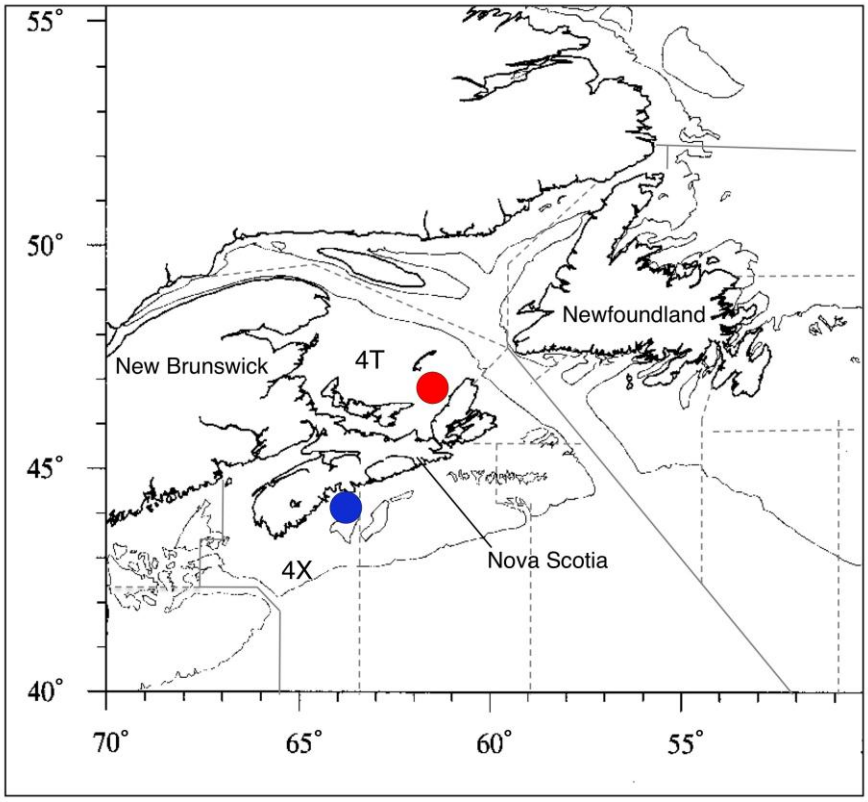


Figure 1: Sampling locations of spawning adults from two populations of Atlantic cod (*Gadus morhua*): 4T (red) and 4X-Sambro (blue). Northwest Atlantic Fisheries Organization division (solid line) and subdivision (dashed line) boundaries are shown in light grey.

### *2.1.2 Common-garden Experiments*

#### *2.1.2.1 Broodstock*

Adult cod from 4T and 4X-Sambro were captured from the wild immediately prior to their spawning seasons in May 2011 and November 2011, respectively, and transported to Dalhousie University for spawning. Sample sizes were 34 and 51 cod from 4T and 4X-Sambro, respectively. Adults were allowed to spawn undisturbed in a 684-$m^3$ pool tank at approximately 8°C and fed dry pellets daily.

*2.1.2.2 Egg Collection And Incubation*

Eggs were sampled approximately five weeks after they were first observed in mesh egg collectors positioned near the surface outflows of the pool tank. Cod are batch-spawners, meaning that females release 5-25% of their eggs at a time during their spawning periods of 3-6 weeks (Chambers & Waiwood 1996; Kjesbu et al. 1996). To increase the probability that a substantial number of families were represented within each spawning group, two batches of fertilized eggs were collected from each population, where each batch consisted of eggs spawned over two consecutive days. Eggs were incubated in 130-litre flow-through tanks at 7°C until hatching.

*2.1.2.3 Larval Rearing*

When nearly all eggs had hatched (day 0 of the experiment), larvae were randomly sampled from each batch and transferred into experimental tanks. Each 30-litre flow-through aquarium contained two 10-litre units, or sub-tanks, in a seawater bath. Larvae were reared in the 10-litre units to mitigate mortality caused by strong currents within the flow-through tanks. Larvae were reared at three temperatures (3°C±1°C, 7°C±1°C and 11°C±1°C) with three (for 4X-Sambro due to an insufficient number of larvae) or four (for 4T) replicate tanks per treatment (Figure 2). 200 larvae were placed in each tank in rotation until a total of 1200 larvae were in each tank (i.e. 600 per unit to a density of 60 larvae per litre). On the day of transfer, all tanks were set to 7°C. The following day (day 1), the water in the tanks was gradually changed to the experimental temperatures over the course of 12 hours.

Larvae were fed rotifers at a density of 4500 prey/litre, three times per day (at approximately 9:00 am, 1:00 pm and 5:00 pm). Larvae were fed Isochrisis-enriched rotifers from days 1-10, Ori-Green-enriched rotifers from days 11-31, a 1:1 mixture of rotifers and *Artemia* from days 32-39 and *Artemia* only from days 40-43. To maintain water quality within the units with minimal disturbance to the larvae, 4 litres of water from the surrounding seawater bath were poured through each unit three times daily

immediately prior to feeding and both the units and tanks were cleaned as needed. Larvae were reared under a light intensity of 2000 lux and water temperatures were monitored daily.

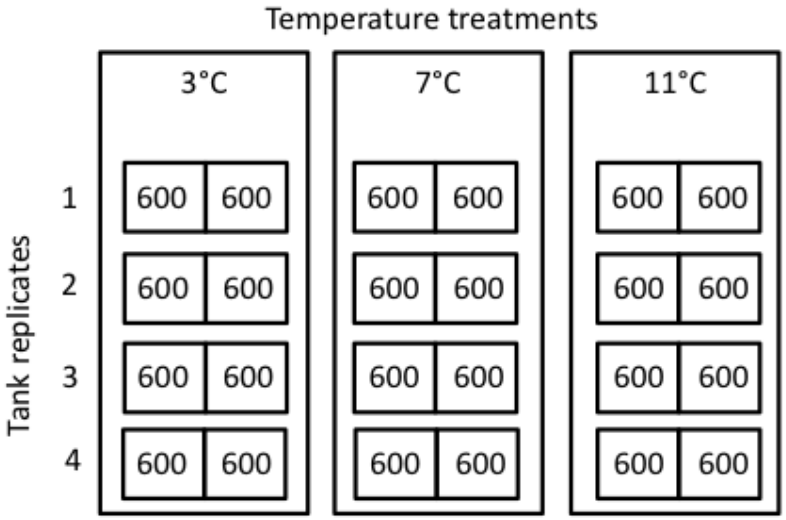


Figure 2: Common-garden experimental design. Larvae were reared in three temperature treatments with four replicate tanks per treatment, each divided into two units containing 600 larvae each.

*2.1.2.4 Collection Of Survival And Growth Data*

On day 0, 60 larvae were randomly sampled from the larvae batches for initial length measurements, hereafter referred to as 'length at hatch'. On days 14, 29, and 43, up to ten larvae from each tank (five from each sub-tank if possible) were sampled for length measurements. Tank and sub-tank number were recorded for day 29 4X-Sambro larvae only. On day 29, the number of larvae in each sub-tank was counted using a hand-held tally counter. The experiments ended on day 43 (i.e. 43 days post hatch, the approximate age of metamorphosis), at which time the number of remaining larvae in each tank was counted. Counting occurred prior to sampling for length measurements. Survival was measured on day 29 and day 43 as the number of larvae alive in each tank, relative to the number alive at day 0.

To obtain length measurements, larvae were euthanized, using ethanol, and individually digitally photographed on a wet petri dish, using AxioVision image analysis software (Zeiss). A ruler was photographed at the start of each series of photographs and was used to calibrate the image analysis software. I measured standard length ("the distance from the tip of the longest jaw to the end of the hypural bone or caudal peduncle"; Kahn et al. 2004), which I interpreted as being from the jaw to the end of the notochord in the absence of hypural bone during the larval stage. Following Hutchings et al. (2007), length-at-day was used as a proxy for growth.

*2.1.2.5 Adult Data Collection*

After spawning was complete, surviving 4X-Sambro adults were anaesthetized, using tricaine methanesulfonate (TMS; Syndel Laboratories Ltd.), to allow for the collection of tail fin-clips (for genotyping), and standard length and weight measurements. At the same time, fin-clips were collected from surviving 4T adults for genotyping. All fin-clips were stored in 95% ethanol at 4°C.

### *2.1.3 Estimating Family Representation*

*2.1.3.1 Microsatellite Genotyping*

To determine the number of families represented in each common-garden experiment and evaluate whether this varied over time, I genotyped 120 larvae that were sampled on day 0 and all larvae that were sampled on day 29 for growth measurements. Larvae were stored in 95% ethanol at 4°C and genotyped at five microsatellite DNA loci (Gmo8, Gmo19, Gmo34, Gmo35, and Tch5), following Hardie et al. (2006). Adults were genotyped at the same five loci, using tissue samples obtained post-spawning and the same methods (Hardie et al. 2006).

*2.1.3.2 Family Reconstruction*

I used both CERVUS v3.0 (Kalinowski et al. 2007) and COLONY v2.0.2.3 (Jones and Wang 2010) to reconstruct the pedigrees of the spawning adults and larvae from each population. CERVUS uses likelihood based on dyads (pairs of individuals) and trios (offspring, parent 1, parent 2) to determine the parentage of each offspring and a simulated parentage analysis to estimate the confidence of the results (Kalinowski et al. 2007). In contrast, COLONY uses a Bayesian approach to calculate likelihood based on the entire pedigree and infers sibship and parentage simultaneously, thereby identifying full-sib families (Jones and Wang 2010). The outputs from both programs were compared and the final pedigrees were viewed using Pedigree Viewer (downloaded at http://www-personal.une.edu.au/~bkinghor/pedigree.htm).

Parental assignment using CERVUS v3.0 (Kalinowski et al. 2007) was performed on day 0 and day 29 larval samples separately. Initially, the proportion of mistyped loci was set to 0.01. This allowed for subsequent manual identification and correction of scoring errors, after which the analyses were performed two more times with the proportion of mistyped loci set to 0.01 and 0.00. The simulated parentage analysis was performed using 10,000 offspring and the actual number of genotyped parents: 37 (0.7255 proportion sampled) for 4X-Sambro and 4 (0.1176 proportion sampled) for 4T. I included parent pairs with the two highest joint LOD scores (the natural log of the overall likelihood ratio) for each offspring in the final parentage analysis. Default settings were used for the remaining parameters. Results were compared among analyses that were repeated with different error rates.

Parental assignment using COLONY v2.0.2.3 (Jones and Wang 2010) was performed on day 0 and day 29 larval samples combined and the genotypes of candidate parents were supplied, providing COLONY with a larger pedigree from which to determine likelihoods. I used the full-likelihood method with medium precision and a random seed and chose to update the allele frequencies as the analyses progressed to mitigate potential bias due to large families. Each analysis consisted of three simultaneous runs to increase the chances of finding the best configuration with the maximum likelihood and obtain more reliable estimates of uncertainty. I used a per locus

error rate of 0.01 and repeated all analyses using short, long, and very long runs to assess whether the maximum likelihood configuration had been reached.

### *2.1.4 Growth Reaction Norms*

All statistical analyses were performed in R (R Development Core Team 2012). A one-way analysis of variance (ANOVA) was conducted on length at hatch to determine whether the size of newly hatched larvae differed among populations. The distribution of lengths differed slightly from a normal distribution, as assessed using a normal quantile-quantile (Q-Q) plot (Appendix B, Figure 1[B1]). However, transformations did not improve normality so the analysis was performed on untransformed data. The variances were fairly homogeneous overall (Figure B2) and between populations (Figure B3).

I constructed thermal reaction norms for larval growth at day 14 and day 29. Due to high mortality among the 4T larvae, there was an insufficient number of 4T larvae on day 43 to obtain reliable length estimates. For each sampling day, I tested for population differences in growth reaction norms using a linear mixed-effects model:

$$\text{length} = \text{population} + \text{temperature} + \text{population} \times \text{temperature} + \text{tank(temperature)} + \varepsilon$$

where tank is a random effect, the remaining terms are categorical fixed effects, and $\varepsilon$ is the error ($\varepsilon \sim N[0,\sigma^2]$). A significant population × temperature interaction (i.e. genotype × environment interaction) indicates a significant difference in the slopes of the reaction norms. *Post hoc* contrasts based on the observed reaction norms were used to determine population-specific levels of plasticity (i.e. temperature effects) and identify significant differences in reaction norm slopes between populations. Results are given with and without a Bonferroni correction for multiple comparisons. Results were considered significant at $\alpha=0.05$ and marginally significant (i.e. uncertain whether different or not) at $\alpha=0.1$. For both ANOVAs, the assumption of normality was not seriously violated (Figures B4 and B9) and there was no evidence of heterogeneity of variances in the data (Figures B5-8 and B10-13).

I evaluated whether sub-tank explained any of the variation in length observed in the 4X-Sambro day 29 larvae by comparing the $AIC_C$ values of linear mixed-effects models with and without sub-tank:

$$\text{length} = \text{temperature} + \text{tank(temperature)} + \varepsilon$$

$$\text{length} = \text{temperature} + \text{tank(temperature)} + \text{sub-tank(tank)} + \varepsilon$$

where temperature is a fixed effect, tank and sub-tank are random effects, and ε is the error.

Reaction norms for growth have been shown to differ at the family level in some fish species (e.g. Chinook salmon [*Oncorhynchus tshawytscha*]*;* Evans et al. 2010). If this is true for cod, there is the potential for families present in high proportions in the experiment to bias the resulting reaction norms. To estimate whether the growth reaction norms might be biased, I performed a two-way ANOVA to compare reaction norms for 4X-Sambro at day 29 based on two different data sets: 1) all available data, and 2) lengths that had been averaged within families within temperatures (i.e. each family only contributed one mean length value to each temperature treatment). The following linear model was used:

$$\text{length} = \text{data set} + \text{temperature} + \text{data set} \times \text{temperature} + \varepsilon$$

where data set and temperature are fixed effects and ε is the error.

I detected small deviations from normality in the data (Figure B14), with a slight bias towards large, positive residuals (Figures B15-18). No other evidence of heterogeneity was observed.

### *2.1.5 Survival Reaction Norms*

Survival at day 43 was too low in the 4T experiment to construct a reliable reaction norm (a total of 10 larvae survived to day 43 across all tanks). Therefore reaction norms were constructed using survival data at day 29 only. I constructed thermal reaction norms for survival for each population, using back-transformed model estimates from a generalized linear model with a quasi-binomial distribution and logit link. The quasi-binomial distribution was necessary to account for overdispersion (dispersion parameter >1) in the data. The estimated dispersion parameter was 17.39. The following model was used:

$$\text{survival} = \text{population} + \text{temperature} + \text{population} \times \text{temperature} + \varepsilon$$

where population, temperature, and their interaction are fixed effects and $\varepsilon$ is the error. I used the same model to test for a population × temperature interaction, except that I used the identity link instead of the logit link. Testing for an interaction on a log scale is influenced not only by the reaction norm slopes but also by their elevations, such that identical slopes on a linear scale can result in significantly different slopes on a log scale if there is a difference in elevation. To use the identity link, I increased survival for all tanks by 1 larva (0.08%) to eliminate zeros in the data set. Deviance tables were used to determine the best model using Chi square tests and the forward stepwise method. Contrasts were used to identify the differences. Results are given with and without a Bonferroni correction for multiple comparisons and were considered significant at $\alpha$=0.05 and marginally significant at $\alpha$=0.1.

For both models I detected no serious deviations from normality (Appendix C, Figure 1 [C1] and C6). 4X-Sambro had a much larger variance than 4T, although this was primarily due to two large residuals at 7°C (Figures C3 and C8). However, the residuals were evenly spread about zero and no other evidence of heterogeneity was observed (Figures C2-5 and C7-10).

## 2.2 Results

### *2.2.1 Estimating Family Representation*

#### *2.2.1.1 Microsatellite Genotyping*

Microsatellite genotypes were successfully obtained for 4X-Sambro day 0 (n=120) and day 29 (n=89) larvae, 4T day 0 (n=30) larvae and 4X-Sambro (n=37) and 4T (n=4) adults. No 4T day 29 larvae were genotyped successfully. 278/280 (99%) genotypes were comprised of all five loci and 2/280 (1%) genotypes were comprised of four loci.

#### *2.2.1.2 Parental Assignments Determined By CERVUS v3.0*

Similar results were obtained when different error rates were used in CERVUS v3.0 (Kalinowski et al. 2007): four and one parental assignment(s) differed for 4X-Sambro day 0 and day 29, respectively, and no assignments differed between the 4T day 0 analyses. As a result, the proportions of larvae that were assigned to known parents were consistent between analyses that used different error rates: 82% for 4X-Sambro day 0, 74-75% for 4X-Sambro day 29, and 13% for 4T day 0. However, the level of confidence associated with these assignments varied for 4X-Sambro (Table 1). Grouping parent-offspring trios into families revealed 17-18 and 14 families in the 4X-Sambro day 0 and day 29 samples, respectively, and one family in the 4T day 0 sample (Table 1).

Table 1: Number of 4X-Sambro and 4T larvae that were assigned to a parent pair using various error rates in CERVUS v3.0 (Kalinowski et al. 2007) and the total number of full-sib families identified by each analysis.

| | 4X-Sambro (Day 0) | | 4X-Sambro (Day 29) | | 4T (Day 0) | |
|---|---|---|---|---|---|---|
| | Error=0.01 | Error=0.00 | Error=0.01 | Error=0.00 | Error=0.01 | Error=0.00 |
| *Confidence Level* | | | | | | |
| >95% | 55 (46%) | 33 (28%) | 51 (57%) | 66 (74%) | 4 (13%) | 4 (13%) |
| >80% | 99 (82%) | 98 (82%) | 66 (74%) | 67 (75%) | 4 (13%) | 4 (13%) |
| Unassigned | 21 (17%) | 22 (18%) | 23 (26%) | 22 (25%) | 26 (87%) | 26 (87%) |
| Total | 120 (100%) | 120 (100%) | 89 (100%) | 89 (100%) | 30 (100%) | 30 (100%) |
| Number of families | 18 | 17 | 14 | 14 | 1 | 1 |

*2.2.1.3 Full-sib Families Determined By COLONY v2.0.2.3*

For all analyses based on COLONY v2.0.2.3 (Jones and Wang 2010), short runs produced slightly different results than long runs and very long runs. However, results from the long and very long runs were identical (4T) or nearly identical (4X-Sambro – one offspring assignment differed), suggesting that the maximum likelihood configuration had been reached. Therefore, the full-sib families as determined by the very long runs in COLONY were compared to the parental assignments made by CERVUS. These results agreed with the exception of one 4X-Sambro offspring (D2-94) that was identified as belonging to a different set of parents using each program. I retained the assignment made by COLONY for this offspring because the genotype is unknown for one of the parents that COLONY identified and CERVUS is unable to consider an adult of unknown genotype as a putative parent. Therefore the results from the very long runs in COLONY are presented here.

Fifteen full-sib families were identified in samples taken from the start of the 4T experiment, with 1-5 offspring in each family. Nine breeding adults contributed to these families: 4 and 5 members of each sex (Table 2; Figure 3). COLONY identified 22 and 20 full-sib families from 4X-Sambro day 0 and day 29, respectively. These families were derived from 7 and 9 (day 0; Table 3; Figure 4) and 7 and 6 (day 29; Table 4; Figure 5) breeding adults of each sex. Collectively, 29 full-sib families (from 10 and 9 adults of

each sex) were sampled from the 4X-Sambro experiment. Family size as a proportion of sample size ranged from 1-49% (mean=5%) for day 0 and 1-36% (mean=4%) for day 29. One family (parents: A-14 and A-39) contributed disproportionately to the 4X-Sambro experiment, producing 49% and 36% of offspring sampled from the day 0 and day 29 samples, respectively.

In summary, at least 15 full-sib families were represented in the 4T experiment, although the true number of families is likely larger than could be detected by the small sample size that was evaluated. At least 29 full-sib families were represented in the 4X-Sambro experiment, with about the same number of families at the beginning and the (near) end of the experiment and one family comprising a large proportion of the larvae.

Table 2: The number of 4T larvae assigned to each full-sib family as determined using COLONY v2.0.2.3 (Jones & Wang 2010). Row and column totals represent the number of larvae in each half-sib family. Parent IDs are given in the first row and the first column, corresponding to the sexes represented by the red and yellow lines in Figure 3, respectively.

| | A-1 | A-3 | *1 | *2 | Total |
|---|---|---|---|---|---|
| A-2 | 4 | 0 | 0 | 0 | 4 |
| #1 | 1 | 1 | 5 | 1 | 8 |
| #2 | 1 | 2 | 1 | 1 | 5 |
| #3 | 5 | 2 | 1 | 1 | 9 |
| #4 | 0 | 2 | 2 | 0 | 4 |
| Total | 11 | 7 | 9 | 3 | 30 |

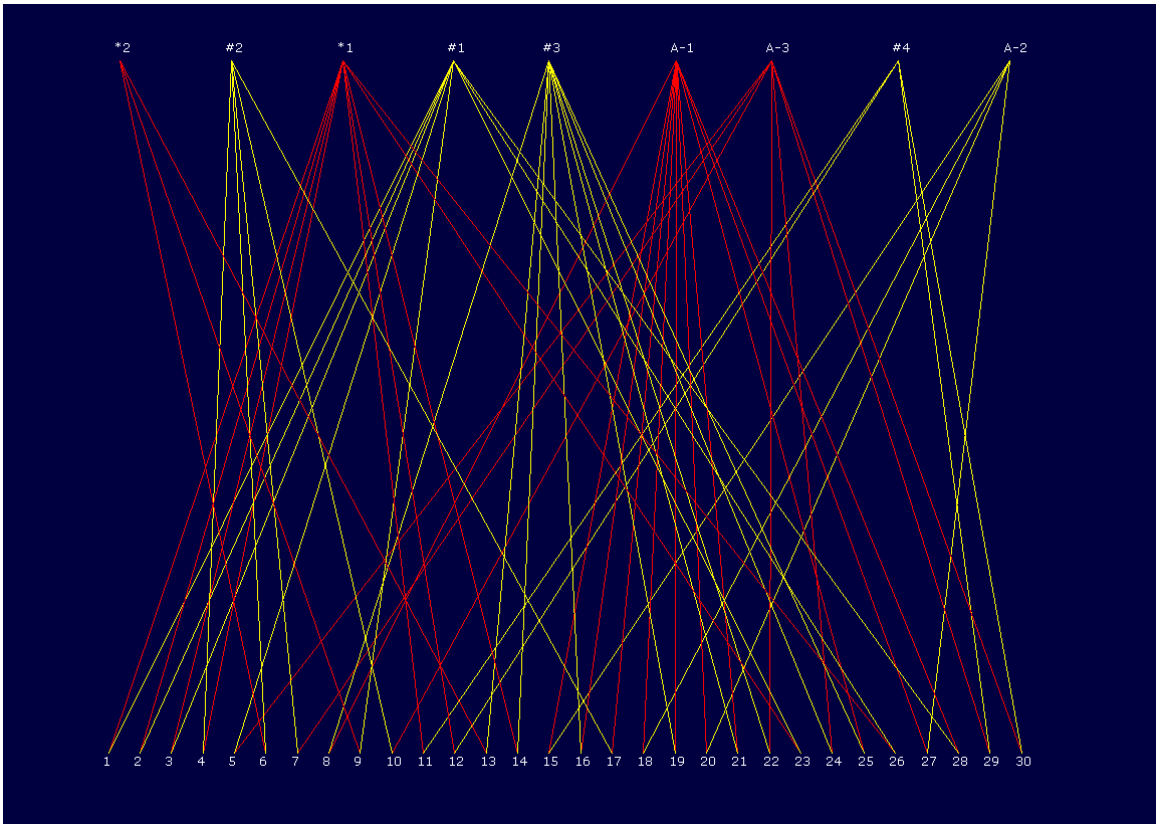


Figure 3: The best pedigree configuration of sampled 4T adults and larvae as determined using COLONY v2.0.2.3 (Jones and Wang 2010), with parent IDs at the top and offspring IDs at the bottom. Red lines indicate adults of one sex and yellow lines indicate adults of the opposite sex.

Table 3: The number of 4X-Sambro larvae sampled on day 0 assigned to each full-sib family as determined using COLONY v2.0.2.3 (Jones & Wang 2010). Row and column totals represent the number of larvae in each half-sib family. Parent IDs are given in the first row and the first column, corresponding to the sexes represented by the red and yellow lines in Figure 4, respectively.

| | A-14 | A-20 | A-27 | A-29 | A-34 | A-36 | A-41 | Total |
|---|---|---|---|---|---|---|---|---|
| A-16 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| A-23 | 4 | 0 | 0 | 0 | 0 | 0 | 1 | 5 |
| A-25 | 8 | 1 | 0 | 1 | 1 | 0 | 1 | 12 |
| A-26 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| A-35 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| A-39 | 59 | 5 | 3 | 0 | 0 | 1 | 2 | 70 |
| #1 | 11 | 4 | 2 | 0 | 0 | 0 | 1 | 18 |
| #2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| #3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Total | 93 | 10 | 7 | 1 | 1 | 1 | 7 | 120 |

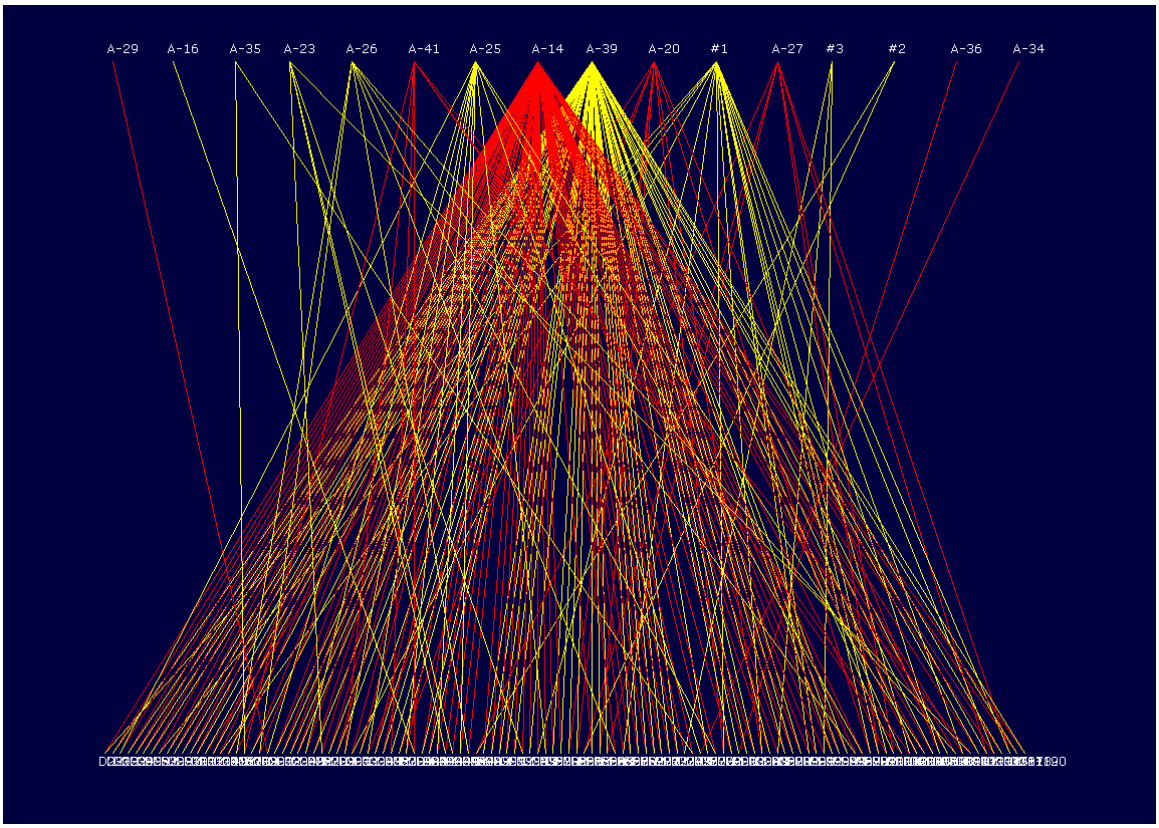


Figure 4: The best pedigree configuration of sampled 4X-Sambro adults and day 0 larvae as determined using COLONY v2.0.2.3 (Jones and Wang 2010), with parent IDs at the top and offspring IDs at the bottom. Red lines indicate adults of one sex and yellow lines indicate adults of the opposite sex.

Table 4: The number of 4X-Sambro larvae sampled on day 29 assigned to each full-sib family as determined using COLONY v2.0.2.3 (Jones & Wang 2010). Row and column totals represent the number of larvae in each half-sib family. Parent IDs are given in the first row and the first column, corresponding to the sexes represented by the red and yellow lines in Figure 5, respectively.

| | A-14 | A-15 | A-20 | A-22 | A-27 | A-31 | A-41 | Total |
|---|---|---|---|---|---|---|---|---|
| A-16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A-23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A-25 | 14 | 0 | 1 | 0 | 2 | 0 | 0 | 17 |
| A-26 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| A-35 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| A-39 | 32 | 2 | 5 | 1 | 2 | 2 | 3 | 47 |
| #1 | 14 | 0 | 1 | 0 | 2 | 1 | 1 | 19 |
| #2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| #3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Total | 63 | 2 | 8 | 1 | 7 | 4 | 4 | 89 |

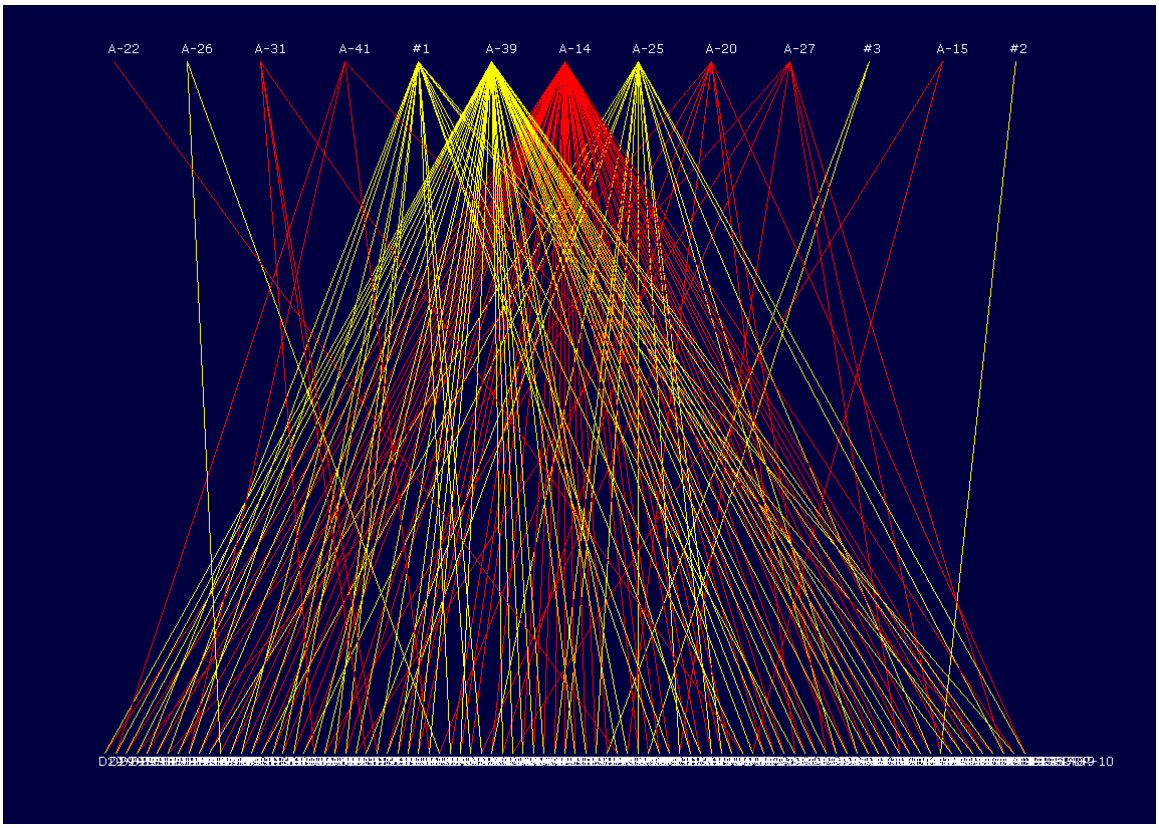


Figure 5: The best pedigree configuration of sampled 4X-Sambro adults and day 29 larvae as determined using COLONY v2.0.2.3 (Jones and Wang 2010), with parent IDs at the top and offspring IDs at the bottom. Red lines indicate adults of one sex and yellow lines indicate adults of the opposite sex.

### *2.2.2 Growth Reaction Norms*

Larval length at hatch differed among populations ($F_1$=136.96; $P$<0.001), with 4X-Sambro larvae (4.92±0.03[SE] mm) being larger than 4T larvae (4.18±0.03 mm). Reaction norms for length at day 14 differed in elevation but not slope (Figure 6), with a significant population effect (F=23.75; $P_{1,189}$<0.001) but no significant temperature effect (F=0.45; $P_{2,189}$=0.638) or interaction between population and temperature (F=1.39; $P_{2,189}$=0.251; Table 5). In contrast, reaction norms for length at day 29 revealed significantly different growth responses to temperature between 4X-Sambro and 4T larvae (Figure 7). Here, the interaction between population and temperature was highly significant (F=21.26; $P_{2,127}$<0.001; Table 5). A contrast analysis revealed that 4T larvae exhibited thermal plasticity for growth, with a ≈ 56% greater length when reared at 11°C (8.84±0.28 mm) compared to 3°C (5.68±0.20 mm), while 4X-Sambro larvae did not show a significant change in growth with temperature (Table 6). The population variation in growth responses was evident in both the lower (3°C-7°C; t=-2.111, $P$=0.018) and upper (7°C-11°C; t=3.869; $P$<0.001) range of temperatures studied, although the difference in slopes at the lower temperature range was not significant after correcting for multiple comparisons (Table 6). The amount of variance explained by the tank effect and residual variance for both growth models are given in Table 7.

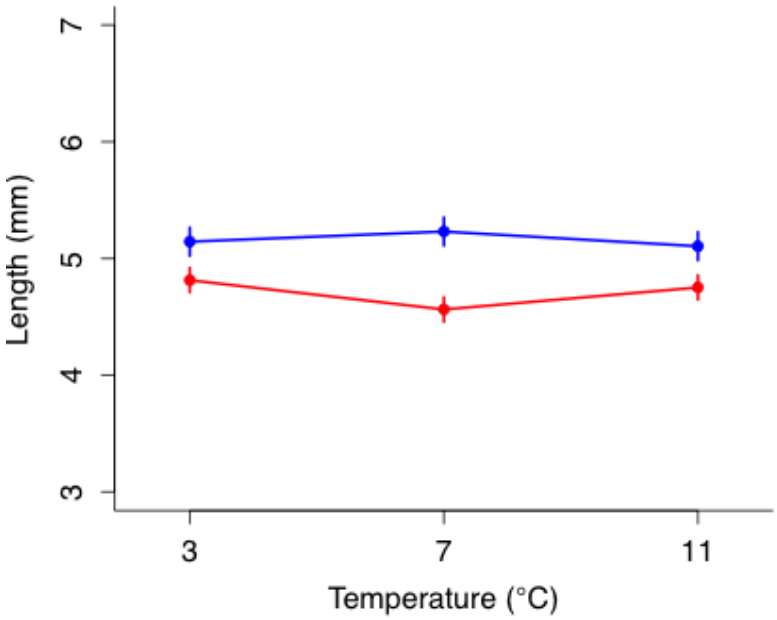


Figure 6: Thermal reaction norms for larval cod length at day 14 (±1 SE) for 4T (red) and 4X-Sambro (blue).

Table 5: Effects of population and temperature on larval length at day 14 and day 29.

| Model term | df | Sum of squares | Mean of squares | F | *P* | |
|---|---|---|---|---|---|---|
| *Day 14* | | | | | | |
| population | 1 | 4.57 | 4.57 | 23.75 | <0.001 | ** |
| temperature | 2 | 0.17 | 0.09 | 0.45 | 0.638 | |
| population × temperature | 2 | 0.54 | 0.27 | 1.39 | 0.251 | |
| *Day 29* | | | | | | |
| population | 1 | 1.59 | 1.59 | 3.18 | 0.077 | * |
| temperature | 2 | 21.23 | 10.61 | 21.16 | <0.001 | ** |
| population × temperature | 2 | 21.33 | 10.66 | 21.26 | <0.001 | ** |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

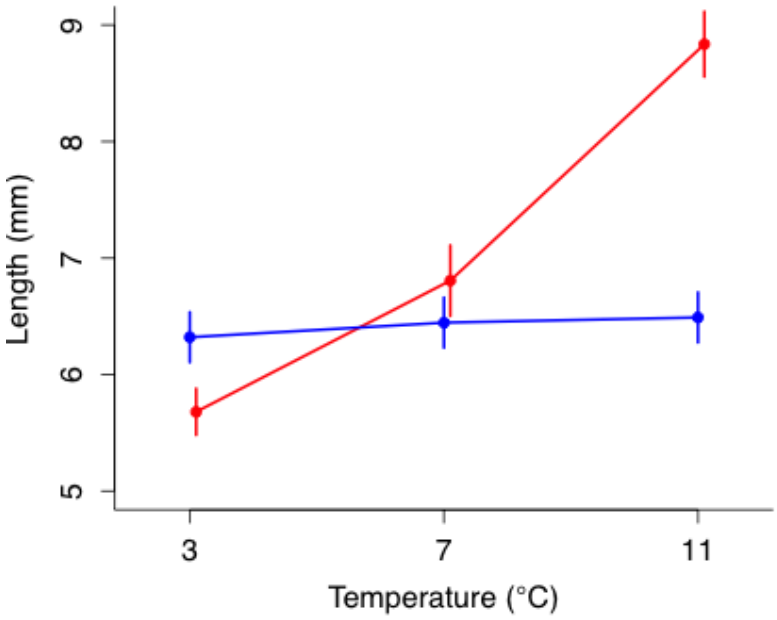


Figure 7: Thermal reaction norms for larval cod length at day 29 (±1 SE) for 4T (red) and 4X-Sambro (blue).

Table 6: Contrast analysis of the effects of population and temperature on larval cod length at day 29. Contrasts are described as A vs. B, where A is the point of contrast.

| Contrast | Estimate | SE | t | *P* | |
|---|---|---|---|---|---|
| *Temperature effects* | | | | | |
| 4X-Sambro at 7°C vs. 4X-Sambro at 3°C | -0.13 | 0.30 | -0.41 | 0.342 | |
| 4X-Sambro at 7°C vs. 4X-Sambro at 11°C | 0.04 | 0.30 | 0.15 | 0.442 | |
| 4T at 7°C vs. 4T at 3°C | -1.13 | 0.36 | -3.10 | 0.006 | ++ |
| 4T at 7°C vs. 4T at 11°C | 2.03 | 0.41 | 4.92 | <0.001 | ++ |
| *Interactions* | | | | | |
| 4X-Sambro slope vs. 4T slope (7°C-3°C) | -1.00 | 0.47 | -2.11 | 0.032 | ** |
| 4X-Sambro slope vs. 4T slope (7°C-11°C) | 1.98 | 0.51 | 3.87 | <0.001 | ++ |

Symbols denote significance at the following levels of α: *=0.1 and **=0.05 (with Bonferroni correction), +=0.1 and ++=0.05 (without Bonferroni correction). A Bonferroni correction for all possible contrasts of interest (n=6) changes the critical *P* values to 0.017 (α=0.1) and 0.008 (α=0.05).

Table 7: Variance explained by the random effect and residual model variance of a linear mixed-effects model of larval cod length at day 14 and day 29.

| Model Term | Variance | Standard deviation |
|---|---|---|
| *Day 14* | | |
| tank | 0.02 | 0.16 |
| residual | 0.19 | 0.44 |
| *Day 29* | | |
| tank | 0.09 | 0.30 |
| residual | 0.50 | 0.71 |

The model of 4X-Sambro larval length at day 29 with sub-tank had a slightly greater $AIC_C$ value (204.1) than the model without sub-tank ($AIC_C$ = 202.1), suggesting it had less support than the more parsimonious model. Thus, the inclusion of sub-tank in the linear model used to compare populations is unlikely to explain any additional variation.

Growth reaction norms for 4X-Sambro at day 29 using 1) all available data, and 2) lengths that were averaged within families within temperatures were not significantly different from one another (F=0.62; $P_{5,117}$=0.688; Table 8). Therefore, the reaction norm for 4X-Sambro was not biased due to the presence of some families that made up a large proportion of the larvae in the experiment.

Table 8: Effects of data set and temperature on larval cod length at day 29 for 4X-Sambro when comparing reaction norms constructed from 1) all available data, and 2) lengths averaged within families within temperatures.

| Model term | df | Sum of squares | Mean of squares | F | *P* |
|---|---|---|---|---|---|
| data set | 1 | 0.00 | 0.00 | 0.00 | 0.977 |
| temperature | 2 | 1.12 | 0.56 | 1.21 | 0.301 |
| data set × temperature | 2 | 0.30 | 0.15 | 0.33 | 0.722 |
| residuals | 117 | 54.01 | 0.46 | - | - |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

### *2.2.3 Survival Reaction Norms*

Thermal reaction norms for survival at day 29 differed significantly between 4T and 4X-Sambro larvae in both elevation ($P$<0.001) and slope ($P$<0.001; Figure 8; Table

9), with 4X-Sambro larvae experiencing higher survival and greater plasticity than 4T larvae. Survival of 4X-Sambro larvae decreased with increases in temperature, with 19% lower survival at 7°C compared to 3°C ($P$<0.001; Table 10). 4X-Sambro survival was reduced by an additional 4% at 11°C, however this decrease was not significant ($P$=0.109; Table 10). Conversely, survival of 4T larvae did not differ between temperature treatments ($P_{3\text{-}7°C}$=0.352, $P_{7\text{-}11°C}$=0.648; Table 10). The thermal responses exhibited by the two populations differed significantly in the lower (3-7°C; $P$=0.001) and marginally in the upper (7-11°C; $P$=0.098) range of temperatures studied, although variation in slopes in the upper range was not significant after correcting for multiple comparisons (Table 10).

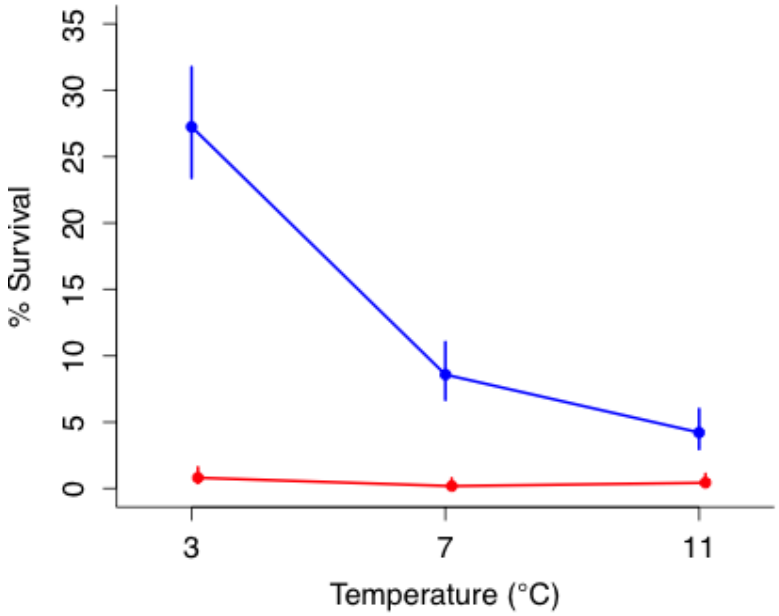


Figure 8: Thermal reaction norms for larval cod survival at day 29 (±1 SE) for 4T (red) and 4X-Sambro (blue).

Table 9: Deviance table of the effects of population and temperature on larval survival at day 29 for 4T and 4X-Sambro cod. *P*-values were obtained from Chi square tests that were used to determine if the model fit improved significantly by sequentially adding population, temperature and their interaction to the null model.

| Model term | df | Deviance | Residual df | Residual deviance | *P* | |
|---|---|---|---|---|---|---|
| null | - | - | 20 | 2774.97 | - | |
| population | 1 | 1819.71 | 19 | 955.26 | <0.001 | ** |
| temperature | 2 | 118 | 17 | 837.26 | 0.312 | |
| population × temperature | 2 | 577.03 | 15 | 260.23 | <0.001 | ** |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

Table 10: Contrast analyses of the effects of population and temperature on larval cod survival at day 29. Contrasts are described as A vs. B, where A is the point of contrast.

| Contrast | Estimate | SE | t | *P* | |
|---|---|---|---|---|---|
| *Temperature effects* | | | | | |
| 4X-Sambro at 7°C vs. 4X-Sambro at 3°C | 13.49 | 3.11 | 4.34 | <0.001 | ++ |
| 4X-Sambro at 7°C vs. 4X-Sambro at 11°C | -3.85 | 2.26 | -1.70 | 0.109 | |
| 4T at 7°C vs. 4T at 3°C | 0.62 | 0.64 | 0.96 | 0.352 | |
| 4T at 7°C vs. 4T at 11°C | 0.25 | 0.53 | 0.47 | 0.648 | |
| *Interactions* | | | | | |
| 4X-Sambro slope vs. 4T slope (7°C-3°C) | -12.87 | 3.17 | -4.06 | 0.001 | ++ |
| 4X-Sambro slope vs. 4T slope (7°C-11°C) | 4.10 | 2.32 | 1.77 | 0.098 | * |

Symbols denote significance at the following levels of α: *=0.1 and **=0.05 (with Bonferroni correction), +=0.1 and ++=0.05 (without Bonferroni correction). A Bonferroni correction for all possible contrasts of interest (n=6) changes the critical *P* values to 0.017 (α=0.1) and 0.008 (α=0.05).

## 2.3 Discussion

In general, I found thermal reaction norms for larval growth and survival to differ between two Atlantic cod populations that experience different thermal environments during the larval stage: relatively warm and variable (4T) and relatively cold and invariant (4X-Sambro) (Marcil 2004; Marcil et al. 2006; Figure A1). Reaction norms for larval length at 29 days post hatch suggest 4T cod larvae have a highly plastic growth response to temperature, growing faster at higher temperatures, whereas there is no evidence of plasticity for growth in the 4X-Sambro cod. The type of response was consistent across the range of temperatures examined, although the slope of the 4T response, and consequently the degree of reaction norm divergence, was greater between

the higher temperature treatments. The opposite pattern of plasticity was true for survival: 4X-Sambro larvae were highly sensitive to temperature, experiencing drastically lower survival in the high-temperature treatments, whereas 4T cod showed similar rates of survival at all temperatures. These patterns of survival were most divergent at lower temperatures.

Interestingly, neither plasticity, nor variation in reaction norm slopes, was observed for length at 14 days post hatch. This lack of differentiation could reflect the similarity in temperatures experienced by 4T and 4X-Sambro cod shortly after spawning (Figure A1). Alternatively, similar thermal responses measured at day 14 may be due to insufficient growth during the first two weeks of life to be manifested by detectable differences between temperature treatments. This explanation is supported by the fact that the change in length from hatch to day 14 was small (0.53±0.51[SD] mm [4T] and 0.24±0.38 mm [4X-Sambro]) relative to the change in length from day 14 to day 29 (up to 4.10±1.31 mm at 11°C [4T] and 1.26±0.78 mm [4X-Sambro]). In addition, Steinarsson and Björnsson (1999) showed that larval growth potential increases steadily over the course of the larval stage.

For those populations that exhibited plasticity for either trait, perhaps the best phenotypes from a fitness perspective were observed at temperatures similar to those typically experienced in the wild. 4T larvae grew the fastest at 11°C, which is in the upper range of typical temperatures experienced two months after initial spawning (Figure A1). Survival of 4X-Sambro larvae was highest at 3°C, which is the mean temperature experienced by these larvae two months after spawning (Figure A1). Presuming that fast growth and high survival confer fitness advantages (Anderson 1988; Hutchings 2011), this variability in reaction norms is consistent with the hypothesis that these populations are adapted to their local thermal regimes.

Further evidence that these divergent responses might represent adaptive variation comes from an examination of how the growth and survival reaction norms co-vary. Survival was maintained at equal (albeit low) levels at all temperatures in the plastic 4T

cod that had the ability to produce many potentially adaptive phenotypes to suit a variety of thermal environments. This may be an example of plasticity for one trait allowing stability of another (Bradshaw 1965). In contrast, survival was severely reduced in 4X-Sambro cod at temperatures outside of the normal range experienced in the wild, suggesting a detrimental consequence to being unable to physiologically adapt to the foreign environments in the short term. The general physiological mechanism behind such a thermal limit has been called oxygen- and capacity-limited tolerance, whereby a mismatch between oxygen supply and demand worsens as temperatures deviate further from the optimum, beyond thermal limits (reviewed in Pörtner 2001, 2002).

The divergent thermal responses observed in the present study might have evolved through a specialist-generalist trade-off whereby 4X-Sambro cod perform very well within a narrow range of cold temperatures at the expense of good performance at temperatures outside their native range. By contrast, 4T cod might have relatively lower maximum performance but perform reasonably well at a wider range of temperatures. The range of temperatures that a population can tolerate can also be described as "thermal windows" (e.g. Pörtner et al. 2008; Pörtner & Peck 2010). Specialist-generalist trade-offs have been used to explain thermal reaction norm variation for growth rate in Atlantic salmon (*Salmo salar*) through selection for particular paralogous trypsin isozymes (Rungruangsak-Torrissen et al. 1998).

Alternatively, selection for energy savings at cold temperatures might explain the narrow thermal window of 4X-Sambro cod. For cold-adapted eurytherms (species that tolerate thermal variability, yet specialize in a cold environment), the baseline energy costs are higher (Lannig et al. 2003). As a result, the thermal window is thought to be as narrow as possible to enable a greater capacity for aerobic enzyme activity and mitochondrial respiration (Pörtner et al. 2008). However, without details of the specific physiological mechanisms associated with various responses, it is not possible to distinguish between specialist-generalist, allocation, or acquisition trade-offs (or a combination thereof) to explain the observed variation because different proximate mechanisms can produce the same adaptive response (Angilletta et al. 2003).

Previous studies of reaction norms in cod have focused on a relatively narrow range of temperatures (e.g. Marcil et al. 2006; Hutchings et al. 2007). The range of temperatures at which phenotypes were measured in the present study encompasses the mean temperatures experienced by both populations in early life as well at least one temperature that each population is not typically exposed to. This permits evaluation of both the portion of the reaction norm that selection has had an opportunity to act on (thus, being potentially adaptive) and that which remains unshaped by natural selection (i.e. non-adaptive). I found that, for those reaction norms that were plastic, both populations exhibited a greater amount of plasticity across the range of temperatures closest to those they experience in the wild. This pattern once again implicates natural selection in shaping these highly sensitive responses.

Outside of the range of environments to which the genotype is adapted, the reaction norm can represent a fundamentally different kind of plasticity that arises from the breakdown of physiological functions in response to environmental stress (Ghalambor et al. 2007). This type of response is usually a passive shift of the phenotype away from the optimum and is consistent with the more shallow slopes observed in the portions of the reaction norms furthest from the optimum phenotypes. Yet another type of non-adaptive plasticity can occur in extreme environments, which is the release of cryptic genetic variation representing the accumulation of neutral mutations in the absence of selection (Rutherford & Lindquist 1998; Rutherford 2000, 2003). I found no evidence of such cryptic variation being expressed, which would manifest in greater variance for phenotypes measured at extreme temperatures (i.e. 3°C for 4T and 11°C for 4X-Sambro). Variation in non-adaptive reaction norms can be useful for predicting the short-term response of a population to environmental stress (see Chapter 4).

Much of these findings are consistent with those of Hutchings et al. (2007), who also found 4T cod larvae to grow faster at 11°C compared to 7°C and to show no effect of temperature on survival. Both studies also detected genetic variation in thermal reaction norms at a relatively small scale of ≈ 600 km. However, unlike Hutchings et al.

(2007) and others (Planque & Frédou 1999; Worm & Myers 2003; Ottersen et al. 2006), I did not find survival to be greater at higher temperatures in cod that experience relatively cold temperatures. In fact, survival of 4X-Sambro cod was much reduced at higher temperatures in my experiment.

This discrepancy could be the product of differing definitions of cold- and warm-water populations. Hutchings et al. (2007) based these classifications on depth-averaged (0-50 m) monthly temperatures for the first two months after initial spawning, in which there was a clear division between cold- (3L and 4X) and warm-water (3Ps and 4T) water populations (3L and 3Ps represent areas from which cod were sampled from Bonavista Bay and Placentia Bay, Newfoundland, respectively; Chapter 3). However, 4X-Sambro does not fit into these categories. Due to its unique fall spawning season and consequent decreasing temperatures during the larval stage, 4X-Sambro groups with the warm-water populations in the first month after initial spawning and the cold-water populations one month later (Figure A1). I classified 4X-Sambro as a cold-water population based on the average temperatures experienced during the first three months after initial spawning. My rationale was that peak spawning occurs over a two month period, therefore a substantial portion of larvae do not reach metamorphosis until the third month after initial spawning. Indeed, 4X-Sambro could be classified as a cold-water population if one only considered temperatures during the second and third month after initial spawning. However, the patterns of plasticity observed for 4X-Sambro cod appear to correspond to a response characteristic of cold-water populations (growth) and a response that is unique, but more similar to that observed for warm-water populations (survival). Therefore, cold- and warm-water classifications, and the mean water temperatures on which they are based, are alone insufficient for explaining population variation in thermal reaction norms.

Regarding the growth responses, one important factor not accounted for by cold- and warm-water generalizations is the manner in which the thermal environment changes with time. Temperature can vary temporally in a stochastic manner (i.e. high variance about monthly mean temperatures) or in a predictable manner associated with seasonality (i.e. differences between monthly means). The Southern Gulf of St. Lawrence

experiences temperatures that are highly variable and seasonal, whereas temperatures on the Southwestern Scotian Shelf are more stable, though still highly seasonal (Figure A1). Plasticity may be adaptive in situations of environmental instability and non-adaptive in stable environments if there is a fitness cost associated with maintaining a plastic response (Via & Lande 1985; Gomulkiewicz & Kirkpatrick 1992; Ghalambor et al. 2007). This hypothesis could explain why 4T cod exhibited plasticity for growth and 4X-Sambro cod did not. An alternative explanation lies in the direction of the seasonal change in temperature experienced by each population, which is positive for 4T and equally negative for 4X-Sambro. 4T cod may have adapted plasticity for growth by taking advantage of the potential for rising temperatures to increase enzyme activity and thus the rate of physiological processes such as metabolism. This ability might not have evolved in 4X-Sambro cod because the larvae experience increasingly colder temperatures in the wild. Therefore, both spatial and temporal variation in temperature may be important in shaping an adaptive norm of reaction in these cod populations.

One potential explanation for the disparity between the survival response of 4X-Sambro compared to the relationship that has been previously documented for cold-water populations (Planque & Frédou 1999; Worm & Myers 2003; Ottersen et al. 2006; Hutchings et al. 2007) relates to the temperatures at which the thermal response is measured relative to the lower thermal limit of a population. The positive relationship between survival and temperature shown in previous studies may only occur at temperatures near the lower thermal limit of cold-water populations. The fact that 4X-Sambro larval survival was highest in the lowest temperature treatment suggests that the lower thermal limit was not approached. If survival had been measured at temperatures below 3°C, the relationship between survival and temperature may have become positive as the lower thermal limit was approached.

Common-garden experiments are one of the most effective means of isolating the genetic basis of phenotypic variation, assuming maternal effects (non-genetic effects of the mother's environment or phenotype on the offspring phenotype; Marshall et al. 2008) are controlled to the greatest extent possible (Conover and Baumann 2009; Hutchings

2011). Ideally, second- or third-generation laboratory cod would be used to eliminate maternal effects. However, the long generation time of Atlantic cod makes this unfeasible. To reduce potential maternal effects that can be exacerbated by stress in breeding females, adults were acclimatized to a common spawning environment for at least five weeks prior to the first egg collection and spawning was allowed to proceed undisturbed in a semi-natural environment. Maternal effects in fishes are mainly caused by variation in egg size, which influences size at hatch (Conover & Schultz 1995; Marshall et al. 2008). Although length at hatch differed between populations, it was not positively associated with growth rate. 4T larvae were smaller at hatch, yet grew at the same rate or faster than 4X-Sambro larvae, depending on the temperature. Therefore it is unlikely that maternal effects are responsible for the observed population differences in growth rate. Length at hatch was positively associated with survival, as 4T larvae consistently had lower rates of survival than 4X-Sambro larvae. It is not known whether maternal effects may have contributed to the high mortality of 4T larvae. Hutchings et al. (2007) found no evidence of maternal effects of growth and survival reaction norms using the same common-garden protocols. Therefore, I interpret the observed variation in growth and survival reaction norms as being the result of genetic differences between populations, although it is not possible to rule out the influence of maternal effects.

The lack of correspondence between larval length at hatch and growth rate observed in the present study differs from previous research on cod larvae from hatchery broodstock in Iceland which showed growth rates (Marteinsdottir & Steinarsson 1997; Steinarsson & Björnsson 1999) and thermal optima for growth (Steinarsson & Björnsson 1999) to increase with length at hatch. Instead, 4T larvae were smaller than 4X-Sambro larvae at hatch and yet demonstrated faster growth and a higher thermal optimum. While growth responses to temperature may be influenced by length at hatch within populations, this relationship might not apply to between-population comparisons due to the more powerful influence of the environment on growth rate and thermal optima. Therefore variation in length at hatch does not explain the shapes of the growth responses in this study.

The experiments described here differed from a classical common-garden experimental design in that populations were studied at different times, as opposed to being raised in the same "garden" at the exact same time. This was necessary due to variation in spawning time between cod populations. With this protocol, there is the potential for environmental variation between the intended common environments, no matter how well these environments are controlled. While this variation may influence the mean trait values of a reaction norm, it would not affect the slopes unless the unknown factor also has an interacting effect with temperature for the traits in question, a scenario that I would argue to be highly unlikely. For this reason, I refrain from interpreting population differences in reaction norm elevations. Support for this line of reasoning stems from the fact that the thermal responses of 4T larvae were consistent between this experiment and that of Hutchings et al. (2007), but overall survival was much lower in this experiment. A direct test of differences between growth reaction norms of 4T larvae obtained from these two studies further supports this conclusion (see Chapter 3).

I found that a small number of spawning adults were responsible for parenting the majority (79%) of offspring in the 4X-Sambro experiment. This is unsurprising given that cod are batch-spawners and, consequently, females release 5-25% of their egg complement intermittently over a 3-6 week spawning period (Chambers & Waiwood 1996; Kjesbu et al. 1996). This reproductive strategy means that a sample of eggs from a spawning aggregation is likely to contain many eggs from some females and few or none from others. Coupled with the fact that male reproductive success is highly skewed towards large body sizes and a propensity for agonistic interactions (Rowe et al. 2008), this reproductive strategy resulted in the majority of experimental offspring belonging to a small number of families. However, the number of families sampled in the 4X-Sambro experiment was comparable to that of previous common-garden work on cod: 29 families compared to 21-71 families in Hutchings et al. (2007). Importantly, I did not find this skewed family representation to bias the resulting growth reaction norms, which suggests a lack of variation in growth response among families. This finding is further evidence

against maternal effects as the source of the reaction norm variation observed in this study.

In summary, I found genetic divergence in plasticity for larval growth and survival between two cod populations across a broader range of thermal environments than had been examined previously. The shapes of the plastic responses and population variation in these shapes suggest that portions of both adaptive and non-adaptive plasticity make up the reaction norms. These patterns suggest cod populations can be adapted to their local environments at a spatial scale of at least ≈ 600 km and allow us to predict how these populations may respond when exposed to temperatures outside of those they normally experience. This study shows that spatial variation in average water temperatures alone may not be sufficient for explaining adaptive divergence in thermal responses among cod populations and suggest possible roles of thermal stability or seasonality for shaping plasticity in cod. Further insight into the role of temperature in promoting adaptive divergence among cod populations requires examination of reaction norms for additional populations that experience a variety of thermal regimes (see Chapter 3).

# Chapter 3: Fine-scale Genetic Variation In Life-history Reaction Norms

## 3.1 Methods

### *3.1.1 Study Populations*

Six common-garden experiments were conducted on five putative Atlantic cod populations (Figure 9): 1) Bay of Fundy (Northwest Atlantic Fisheries Organization [NAFO] division 4X), 2) Southwestern Scotian Shelf near Sambro, Nova Scotia (NAFO division 4X), Southern Gulf of St. Lawrence (NAFO division 4T), Bonavista Bay, Newfoundland (NAFO division 3L), and Placentia Bay, Newfoundland (NAFO division 3Ps). Cod from these areas will be referred to by their NAFO divisions throughout the text. The two groups from the 4X division will be referred to as 4X-Bay of Fundy (or 4X-BOF) and 4X-Sambro. Common-garden experiments on 4X-Bay of Fundy, 4T, 3L, and 3Ps cod were conducted by Hutchings et al. (2007) from 2002-2003. Further experiments were carried out on 4X-Sambro and 4T cod from 2011-2012 (see Chapter 2 for descriptions of these populations). The two experiments using 4T cod will be referred to as 4T-2003 and 4T-2011.

Cod from the Bay of Fundy are from one of what is likely to be multiple spawning components in the Southwestern Scotian Shelf (NAFO division 4X), based in part on spatial differences in spawning times (Nov-Dec: 4X-Sambro [Chapter 2]; Jan-Mar; outer Bay of Fundy [Hutchings et al. 2007]). Migration of Bay of Fundy cod is limited to the habitation of deeper water during the winter months (ICES 1994). It is also during the winter that peak spawning season occurs, from February to March. Because of the winter spawning period, Bay of Fundy larvae experience relatively cold and stable temperatures, with average monthly temperatures ranging from 3±2(SD)°C in February to 5±2°C in May (R. Oomen, unpublished analyses; see Appendix A, Figure 1 [A1] for details). Bay of Fundy cod are differentiated at neutral microsatellite markers from other

spawning components within the 4X division: Browns Bank and Georges Bank (Ruzzante et al. 1998). However, microsatellite markers failed to detect significant neutral divergence between cod from the 4X, 4T, and 3Ps divisions (Hardie et al. 2006).

Cod from Bonavista Bay, Newfoundland (NAFO division 3L), were collected from the coastal component of the Northern Newfoundland cod stock complex (NAFO divisions 2J3KL) that inhabits inshore waters during the summer. The peak spawning period occurs in early June (Myers et al. 1993). As a consequence, 3L larvae experience relatively warm temperatures, increasing from 1±1°C to 6±2°C from May to August (Figure A1).

Placentia Bay cod comprise a coastal component of the St. Pierre Bank cod stock (NAFO division 3Ps). These cod are thought to inhabit Placentia Bay for the majority of the year although they may intermix with adjacent 3L cod because of limited adult migration out of the bay (Lawson and Rose 2000). Cod in Placentia Bay spawn from March to August (DFO 2003), with peak spawning occurring in May (Myers et al. 1993). 3Ps larvae experience relatively warm temperatures ranging from 3±1°C to 9±2°C from May to August (Figure A1).

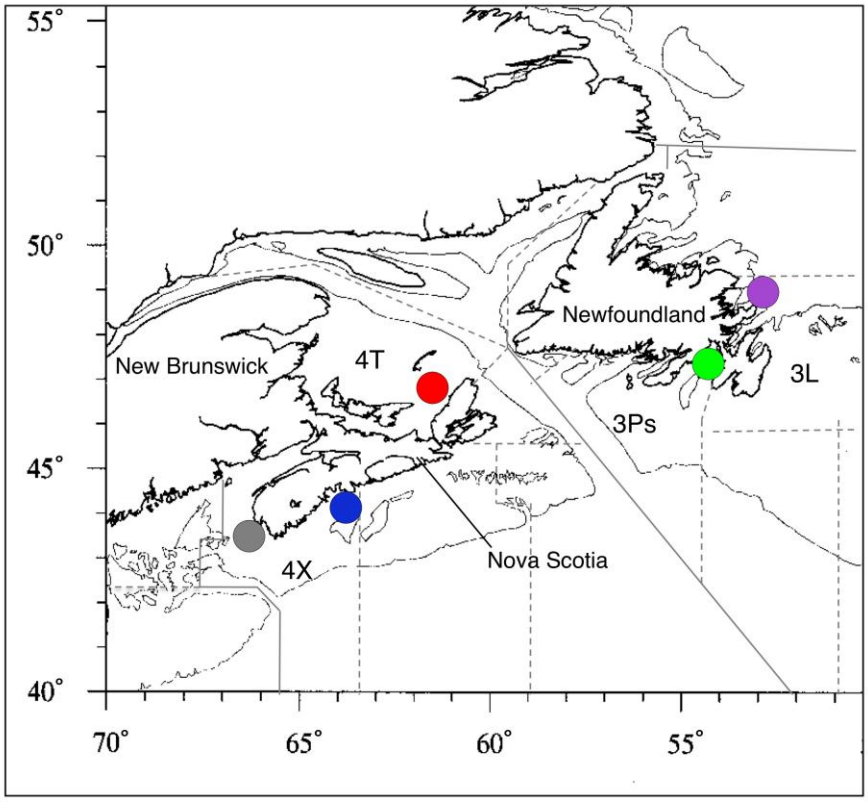


Figure 9: Sampling locations of spawning adults from study populations of Atlantic cod (*Gadus morhua*): 3L (purple); 3Ps (green); 4T (red); 4X-Bay of Fundy (grey); and 4X-Sambro (blue). Northwest Atlantic Fisheries Organization division (solid line) and subdivision (dashed line) boundaries are shown in light grey.

### *3.1.2 Common-garden Experiments*

Common-garden experimental protocols were similar to those described in Chapter 2 (see Hutchings et al. [2007] for details). Procedural and other notable differences between the methods in Chapter 2 and those used by Hutchings et al. (2007) are highlighted in Table 11. Briefly, the methods are as follows.

Wild-caught adults from 4X-Bay of Fundy, 4T, 3L, and 3Ps were obtained during (3L only) or immediately prior to their breeding seasons (see Table 12 for information on broodstock collection locations and dates). Adult cod spawned undisturbed either at Dalhousie University (4X-Bay of Fundy and 4T) or at the Oceans Sciences Centre at

Memorial University of Newfoundland (3L and 3Ps). All common-garden experiments took place at the Ocean Sciences Centre, to which fertilized eggs from Dalhousie University had been transported. Larvae were reared in 30-litre flow-through aquaria at two temperatures (7°C±1°C and 11°C±1°C) with 4 tank replicates per treatment. Length at hatch was measured for all populations. Length at 29 days post hatch was used as a proxy for growth. Survival was quantified as the mean number of larvae alive in each tank at day 43, relative to the number alive at day 0.

Table 11: Summary of differences between spawning and common-garden experiments described in Chapter 2 and those in Hutchings et al. (2007).

| Method or characteristic | Chapter 2 | Hutchings et al. (2007) |
|---|---|---|
| Number of spawning adults | 34-51 | 54-77 |
| Number of families represented | 15 (4T) and 29 (4X-Sambro) | 44 (3Ps), 31 (4T), 21 (4X-Bay of Fundy), and 71 (3L) |
| Timing of egg collection | 5 weeks after eggs were first observed in collectors | 2 weeks after eggs were first observed in collectors |
| Number of egg batches sampled | 2 | 4 |
| Tank set-up | Two 10-litre units in a flow-through seawater bath | 30-litre flow-through aquaria |
| Initial larval density | 60 larvae per litre | 40 larvae per litre |
| Number of tank replicates | 3 (4X-Sambro) or 4 (4T) | 4 |

Table 12: Collection locations and dates of capture of study populations.

| Population | Collection location | Month and year of capture |
|---|---|---|
| 4T | Southern Gulf of St. Lawrence, NS<br>47°N, 61°W | May 2003, May 2011 |
| 4X-Sambro | Scotian Shelf near Sambro, NS<br>44°25'N, 63°30'W | November 2011 |
| 4X-Bay of Fundy | Scotian Shelf<br>44°N, 66°W | January 2002 |
| 3L | Bonavista Bay, NL<br>49°N, 53°W | June 2003 |
| 3Ps | Placentia Bay, NL<br>47.5°N, 54°W | April 2002 |

### *3.1.3 Estimating Family Representation*

The number of families represented in each experiment was 71, 44, 31, 21, and 29 for 3L, 3Ps, 4T, 4X-Bay of Fundy, and 4X-Sambro, respectively. (See Chapter 2 for details on how the number of families was determined for 4X-Sambro.) Although Hutchings et al. (2007) employed methods similar to those described in Chapter 2 for the remaining populations (also see Hardie et al. 2006), they only genotyped larvae sampled at the beginning of the common-garden experiments. Further, the authors used an additional two microsatellite loci (Gmo3 and Mae9) and the program PAPA v.2.0 (Duchesne et al. 2002) for parental assignment.

### *3.1.4 Growth Reaction Norms*

All statistical analyses were conducted in R (R Development Core Team 2012). I performed a one-way analysis of variance (ANOVA) on length at hatch for all six common-garden experiments to determine whether initial larval size differed among populations. *Post hoc* contrasts were used to determine where differences exist. The length at hatch data were approximately normally distributed, as assessed using a normal quantile-quantile (Q-Q) plot (Appendix D, Figure 1 [D1]), although there was a slight excess of large, negative residuals. Variances appeared to be homogeneous overall (Figure D2) and between populations (Figure D3).

I compared growth reaction norms obtained from common-garden experiments that were carried out over a span of 10 years. To ensure that variation in reaction norms was attributable to population differences and not temporal differences, I constructed reaction norms for the two experiments involving 4T cod that were carried out eight years apart (4T-2003 and 4T-2011). I used a linear mixed-effects model:

$$\text{length} = \text{experiment} + \text{temperature} + \text{experiment} \times \text{temperature} + \text{tank(temperature)} + \varepsilon$$

where tank is a random effect, the remaining terms are fixed effects, and ε is the error ($\varepsilon \sim N[0,\sigma^2]$). A two-way ANOVA revealed that, although the elevations (i.e. mean trait values) of the reaction norms differed between 4T-2003 and 4T-2011 (F=5.94; $P_{1,82}$=0.017), the slopes did not (F=0.957; $P_{1,82}$=0.331; Table 13; see Table 14 for the amount of variance explained by the tank effect and residual variance). Thus, comparing reaction norm slopes between experiments carried out over this time frame is unlikely to be confounded by temporal variation, although the same cannot be said for reaction norm elevations. Because the mean trait values differed between the 4T experiments, the data could not be combined. Further, higher mortality was observed in the 4T-2011 experiment, which resulted in an insufficient sample size for constructing reaction norms for survival at day 43. For these reasons, only the 4T-2003 experiment was included in the analyses described below.

Table 13: Effects of experiment and temperature on larval cod length at day 29 for experiments conducted using 4T larvae in 2003 and 2011.

| Fixed effects | df | Sum of squares | Mean of squares | F | *P* | |
|---|---|---|---|---|---|---|
| experiment | 1 | 2.42 | 2.42 | 5.94 | 0.017 | ** |
| temperature | 1 | 10.91 | 10.91 | 26.74 | <0.001 | ** |
| experiment × temperature | 1 | 0.39 | 0.39 | 0.96 | 0.331 | |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

Table 14: Variance explained by the random effect and residual model variance of a linear mixed-effects model of 4T-2003 and 4T-2011 larval cod length at day 29.

| Model Term | Variance | Standard deviation |
|---|---|---|
| tank | 0.22 | 0.47 |
| residual | 0.41 | 0.64 |

I constructed thermal reaction norms for growth for all populations, using a linear mixed-effects model:

$$\text{length} = \text{population} + \text{temperature} + \text{population} \times \text{temperature} + \text{tank(temperature)} + \varepsilon$$

where tank is a random effect, the remaining terms are fixed effects, and ε is the error. Based on the resulting reaction norms, I performed *post hoc* contrasts to determine whether observed differences between specific populations were significant. Results are given with and without a Bonferroni correction for multiple comparisons and were considered significant at $\alpha$=0.05 and marginally significant at $\alpha$=0.1.

For both ANOVAs, I assessed whether the length measurements were normally distributed by plotting the residuals (Figures D4 and D9) and detected no substantial deviations from normality. I also plotted the model residuals for each level of each factor and their interactions (Figures D5-8 and D10-13) and I found no evidence for heterogeneity.

To evaluate whether variation in density among tanks attributable to differential survival may have contributed to tank effects observed in the growth model, I examined whether there was a relationship between the magnitude and direction of random effects and tank density. I used percent survival on day 43 as a proxy for density, as this was the only day for which survival data were available for all populations. Given that a plot of random effect size against survival revealed no pattern of association (Figure 10), differential tank density was considered to be not responsible for variation attributable to differences among tanks.

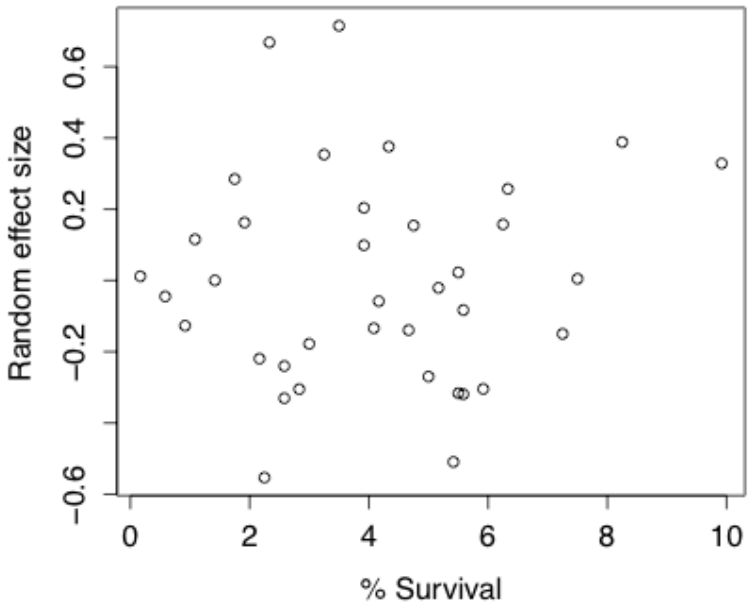


Figure 10: Plot of random effect size for model of larval cod length at day 29 as a function of tank survival at day 43.

### *3.1.5 Survival Reaction Norms*

I constructed thermal reaction norms for survival at day 43 for each population, using back-transformed model estimates from a generalized linear model with a quasi-binomial distribution and logit link:

$$\text{survival} = \text{population} + \text{temperature} + \text{population} \times \text{temperature} + \varepsilon$$

where population, temperature, and their interaction are fixed effects and ε is the error. To test for a population × temperature on the original scale (as opposed to the log scale, in which slope is confounded by elevation), I used the same model as above except with the identity link instead of the logit link. Deviance tables were used to determine the best model using Chi square tests and the forward stepwise method. I then used contrasts to identify which reaction norms differed. Results are given with and without a Bonferroni correction for multiple comparisons and were considered significant at $\alpha=0.05$ and marginally significant at $\alpha=0.1$.

For both models, I detected no serious deviations from normality (Appendix E, Figure 1 [E1] and E6). The variances differed slightly between populations (Figures E3 and E8). However, the residuals were all evenly spread about zero and no other evidence of heterogeneity was observed (Figures E2-5 and E7-10).

## 3.2 Results

### *3.2.1 Growth Reaction Norms*

Larval length at hatch differed among populations ($F_5$=117.13; $P$<0.001), with larvae from the 4X division generally being larger than larvae from the remaining divisions (Table 15). I found substantial population variation in thermal responses for length at day 29 (Figure 11), manifested by a significant population × temperature interaction (F=4.78; $P_{4,261}$=0.001; Table 16; see Table 17 for the amount of variance explained by the tank effect and residual variance). Plasticity in response to temperature was evident in all populations except for 4X-Sambro and was such that larvae grew faster in the high temperature treatment (Table 18). In contrast, growth of 4X-Sambro larvae did not differ between temperature treatments (t=0.13, $P$=0.450). The differences in slopes between 4X-Sambro and the remaining populations were significant or marginally significant after correcting for multiple comparisons except for 3L, which was significant before the correction (Table 19). Among the populations that exhibited plasticity, the magnitudes of the responses were similar (Figure 11). The uncorrected results suggest that the slope of the response of 3Ps larvae was steeper than 3L (t=1.79, $P$=0.043) and marginally steeper than 4T (t=-1.33, $P$=0.097;Table 19). However, these differences were not significant after correcting for multiple comparisons.

Table 15: Larval lengths at hatch for six common-garden experiments.

| Population | Mean length | Standard error |
|---|---|---|
| 3L | 4.41 | 0.04 |
| 3Ps | 4.35 | 0.03 |
| 4T-2003 | 4.52 | 0.03 |
| 4T-2011 | 4.18 | 0.03 |
| 4X-Bay of Fundy | 5.05 | 0.03 |
| 4X-Sambro | 4.92 | 0.03 |

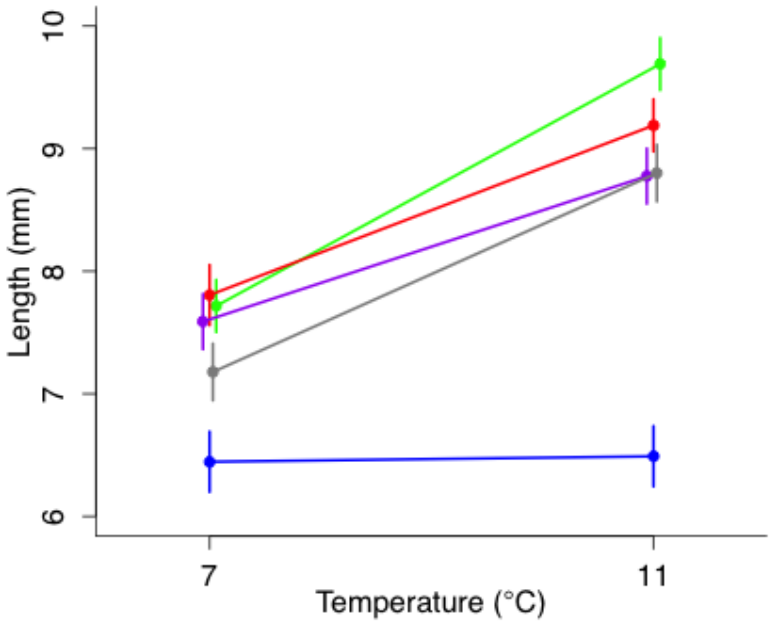


Figure 11: Thermal reaction norms for larval cod length at day 29 (±1 SE) for 3L (green), 3Ps (purple), 4T (red), 4X-Bay of Fundy (grey) and 4X-Sambro (blue).

Table 16: Effects of population and temperature on larval cod length at day 29.

| Fixed effects | df | Sum of squares | Mean of squares | F | *P* | |
|---|---|---|---|---|---|---|
| population | 4 | 36.74 | 9.19 | 28.38 | <0.001 | ** |
| temperature | 1 | 25.92 | 25.92 | 80.07 | <0.001 | ** |
| population × temperature | 4 | 6.19 | 1.55 | 4.78 | 0.001 | ** |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

Table 17: Variance explained by the random effect and residual model variance of a linear mixed-effects model of larval cod length at day 29.

| Model Term | Variance | Standard deviation |
|---|---|---|
| tank | 0.15 | 0.39 |
| residual | 0.32 | 0.57 |

Table 18: The effect of temperature on larval cod length at day 29 for five cod populations, where the estimate represents the change in length from 7°C to 11°C.

| Population | Estimate | SE | t | *P* | |
|---|---|---|---|---|---|
| 3L | 1.19 | 0.32 | 3.72 | <0.001 | ** |
| 3Ps | 1.97 | 0.30 | 6.54 | <0.001 | ** |
| 4T | 1.38 | 0.33 | 4.25 | <0.001 | ** |
| 4X-BOF | 1.62 | 0.33 | 4.95 | <0.001 | ** |
| 4X-Sambro | 0.04 | 0.35 | 0.13 | 0.450 | |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

Table 19: Pairwise population contrasts of the effect of temperature on larval cod length at day 29. Estimates (± SE) are given above the diagonal and *P*-values are given below the diagonal. The point of contrast is the row header for the estimates and the column header for the P values.

| | 3L | 3Ps | 4T | 4X-BOF | 4X-Sambro |
|---|---|---|---|---|---|
| 3L | - | 0.79(±0.44) | 0.20(±0.46) | 0.44(±0.46) | -1.14(±0.47) |
| 3Ps | 0.043 ++ | - | -0.59(±0.44) | -0.35(±0.45) | -1.93(±0.46) |
| 4T | 0.336 | 0.097 + | - | 0.24(±0.46) | -1.34(±0.48) |
| 4X-BOF | 0.177 | 0.219 | 0.308 | - | -1.58(±0.48) |
| 4X-Sambro | 0.012 ++ | <0.001 ** | 0.004 * | 0.002 ** | - |

Symbols denote significance at the following levels of α: *=0.1 and **=0.05 (with Bonferroni correction), +=0.1 and ++=0.05 (without Bonferroni correction). A Bonferroni correction for all possible contrasts of interest (n=15) changes the critical *P* values to 0.007 (α=0.1) and 0.003 (α=0.05).

### *3.2.2 Survival Reaction Norms*

Significant variation in survival reaction norm slopes was observed between populations (*P*<0.001; Figure 12; Table 20). 4X-Bay of Fundy larvae exhibited a high degree of plasticity with a significant positive relationship between survival and temperature (*P*<0.001; Table 21) and 2.5 times greater survival in the high-temperature treatment. The opposite response was observed in 4X-Sambro larvae with survival in the

low-temperature treatment being more than three times greater than survival in the high-temperature treatment, although this effect was not significant after correcting for multiple comparisons ($P$=0.009; Table 21). Several populations (3L, 3Ps, and 4T) exhibited no significant plasticity (Table 21). The slopes of these populations were significantly (3Ps and 4T) or marginally significantly (3L) different from that of 4X-Bay of Fundy and significantly (3L and 3Ps) or marginally significantly (4T) different from 4X-Sambro (Table 22). However, none of these differences were significant after a Bonferroni correction. Only the responses of 4X-Bay of Fundy and 4X-Sambro larvae were significantly different after correcting for multiple comparisons ($P$<0.001; Table 22).

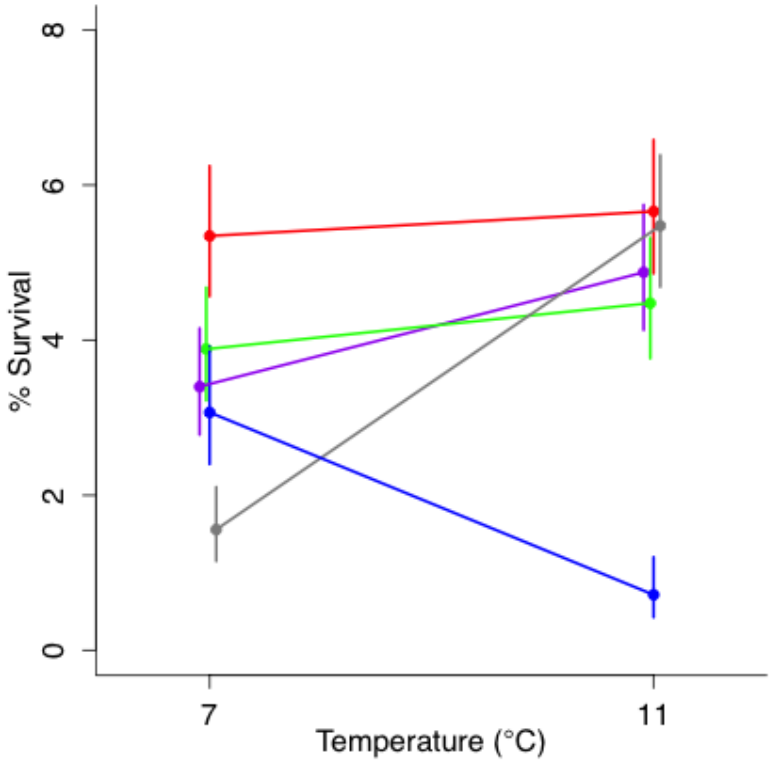


Figure 12: Thermal reaction norms for larval cod survival at day 43 (±1 SE) for 3L (green), 3Ps (purple), 4T (red), 4X-Bay of Fundy (grey) and 4X-Sambro (blue).

Table 20: Deviance table of the effects of population and temperature on larval survival at day 43. *P*-values were obtained from Chi square tests that were used to determine if the model fit improved significantly by sequentially adding population, temperature and their interaction to the null model.

| Model term | df | Deviance | Residual df | Residual deviance | *P* | |
|---|---|---|---|---|---|---|
| null | | | 37 | 564.49 | - | |
| population | 4 | 163.42 | 33 | 401.07 | 0.009 | ** |
| temperature | 1 | 14.09 | 32 | 386.98 | 0.275 | |
| population × temperature | 4 | 179.43 | 28 | 207.55 | <0.001 | ** |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

Table 21: The effect of temperature on larval cod survival at day 43 for five cod populations, where the estimate represents the change in survival from 7°C to 11°C.

| Population | Estimate | SE | t | *P* | |
|---|---|---|---|---|---|
| 3L | 0.01 | 0.01 | 1.40 | 0.173 | |
| 3Ps | 0.01 | 0.01 | 0.56 | 0.581 | |
| 4T | 0.00 | 0.01 | 0.26 | 0.794 | |
| 4X-BOF | 0.04 | 0.01 | 4.04 | <0.001 | ++ |
| 4X-Sambro | -0.02 | 0.01 | -2.81 | 0.009 | ** |

Asterisks denote significance at the following levels of α: * = 0.1, ** = 0.05.

Table 22: Pairwise population contrasts of the effect of temperature on larval cod survival at day 43. Estimates (± SE) are given above the diagonal and *P*-values are given below the diagonal. The point of contrast is the row header for the estimates and the column header for the P values.

| | 3L | 3Ps | 4T | 4X-BOF | 4X-Sambro |
|---|---|---|---|---|---|
| 3L | - | -0.88(±1.50) | -1.16(±1.60) | 2.44(±1.43) | -3.83(±1.35) |
| 3Ps | 0.560 | - | -0.28(±1.60) | 3.32(±1.44) | -2.95(±1.35) |
| 4T | 0.475 | 0.864 | - | 3.60(±1.54) | -2.67(±1.46) |
| 4X-BOF | 0.099 * | 0.028 ** | 0.027 ** | - | -6.27(±1.28) |
| 4X-Sambro | 0.008 ** | 0.038 ** | 0.079 * | <0.001 ++ | - |

Symbols denote significance at the following levels of α: *=0.1 and **=0.05 (with Bonferroni correction), +=0.1 and ++=0.05 (without Bonferroni correction). A Bonferroni correction for all possible contrasts of interest (n=15) changes the critical *P* values to 0.007 (α=0.1) and 0.003 (α=0.05).

## 3.3 Discussion

I found variation in thermal reaction norms for larval growth and survival among five populations of Atlantic cod in the Northwest Atlantic. Four populations (3L, 3Ps, 4T

and 4X-Bay of Fundy) presented a highly plastic growth response to temperature, growing faster in warmer water, whereas there is no evidence of plasticity for growth in 4X-Sambro cod. The magnitude of change may also differ slightly among the plastic growth responses, with 3Ps exhibiting the largest response, however further study will be needed to confirm the significance of this subtle variation. An even greater variety of thermal responses were observed for survival, with cod from the 4X-Bay of Fundy population experiencing drastically higher survival in warmer water, while the opposite response was observed for 4X-Sambro. Survival of 3L, 3Ps, and 4T larvae was not affected by temperature.

Faster larval growth is believed to be adaptive by shortening the larval stage, thus reducing the high risk of mortality associated with early life (Anderson 1988; Steinarsson & Björnsson 1999). Therefore it is not surprising that faster growth in the high temperature treatment experiment was associated with either maintained or improved survival. 4X-Sambro was the only population that did not exhibit plasticity for growth and it was the only population to experience lower survival in the high temperature treatment.

Considering the growth and survival reaction norms together, three groups emerge based on the types of responses they exhibit to increased temperature: faster growth and enhanced survival (4X-Bay of Fundy), faster growth and equal survival (3L, 3Ps, and 4T), and equal growth and decreased survival (4X-Sambro). These groups of populations could also be characterized as winter-spawning, spring-spawning, and fall-spawning, respectively, each of which experiences a different thermal environment during the larval stage (Figure A1). This association between thermal reaction norms and the timing of the spawning season raises the question as to the specific thermal mechanism responsible for shaping these diverse responses.

As discussed in Chapter 2, the mean temperature experienced during the larval stage is insufficient for explaining the observed reaction norm variation, particularly because it fails to explain the unique thermal response of 4X-Sambro. Instead, thermal

instability was suggested as a possible mechanism by which the high levels of plasticity for growth observed in 4T larvae evolved. However, the remaining three populations exhibiting plastic growth responses experience similar levels of thermal stability as 4X-Sambro (see the standard errors in Figure A1), which is non-plastic for growth. Therefore, thermal stability does not seem sufficient to account for the patterns of variation observed in 3L, 3Ps, and 4X-Bay of Fundy.

The third mechanism proposed in Chapter 2 to explain the observed reaction norm variation is seasonal changes in temperature during the larval stage. Mean monthly temperatures experienced during the larval stage increase for spring- and winter-spawning populations and decrease for the fall-spawning 4X-Sambro cod (Figure A1). The patterns of plasticity for growth and survival suggest a strong adaptation to seasonal warming in 3L, 3Ps, 4T, and 4X-Bay of Fundy, whereby the effect of temperature on survival is stronger in those populations that experience relatively colder temperatures overall. This observation is consistent with previous findings that cold-water populations experience increasing survival with temperature (Planque & Frédou 1999; Worm & Myers 2003; Ottersen et al. 2006). 4X-Sambro also experiences relatively cold temperatures, however they decrease during the larval stage. As a result, 4X-Sambro cod may not have experienced the selective pressures necessary to shape an adaptive norm of reaction for growth to higher temperatures. Coupled with the fact that growth rate at lower temperatures is limited by thermodynamic constraints on enzymatic activity (Clarke & Fraser 2004), the result is a lack of plasticity for growth. Further, the negative impact of increasing temperatures on survival represents a constraint on the ability of 4X-Sambro larvae to maintain basic physiological functions in response to environmental stress. Based on the corresponding survival response, the non-plastic reaction norm for growth could be considered to be maladaptive at these high temperatures. Therefore, even though the thermal response of 4X-Sambro is not necessarily adaptive at high temperatures, it might be the result of being adapted to lower temperatures that the larvae typically experience in the wild and the trade-off between having high performance in native environments at the expense of low performance in others (i.e. local adaptation). It is of note that these patterns of differentiation in plasticity could also be characterized by

the mean temperatures experienced near the end of the larval stage at three months after initial spawning, whereby populations that experience mean temperatures >3°C exhibit a plastic growth response and greater or equal survival at higher temperatures and those that experience temperatures <3°C and show no growth response and decreasing survival with temperature.

Given the well-established role of ambient temperature in regulating poikilotherm metabolism and a broad array of physiological processes (Clarke & Fraser 2004), it is unsurprising that temperature has emerged as an important driver of local adaptation in marine fishes (e.g. Fangue et al. 2006; Bradbury et al. 2010; Baumann & Conover 2011; Hice et al. 2012). In addition to the thermal reaction norm variation detected by Marcil et al. (2006) and Hutchings et al. (2007), common-garden experiments support genetic differences in the effects of temperature on condition factors in juvenile cod, including variation in plasticity of the hepatosomatic index, i.e. liver weight as a function of body weight (Purchase & Brown 2001). Evidence of temperature-driven local adaptation in cod has also derived from single nucleotide polymorphisms (SNPs) associated with ocean temperature (Nielsen et al. 2009; Bradbury et al. 2010). Particularly compelling evidence of the pervasiveness of thermal adaptation in cod comes from Bradbury et al. (2010), who showed clinal variation in SNP allele frequencies at temperature-associated genes throughout the North Atlantic.

I found small-scale genetic variation in thermal reaction norms between two cod spawning components within the 4X management division. Spawning cod from 4X-Sambro and 4X-Bay of Fundy were collected ≈ 250 km apart. However, the ranges occupied by these spawning groups are not known and may even overlap. Therefore the spatial scale of adaptive divergence is likely smaller than 250 km. This is the smallest spatial scale at which genetic variation in traits for which the adaptive significance is known has been detected across open waters in a marine fish, that is, waters that are not physically separated by land in some manner, as along coastal Norway (Olsen et al. 2008). That this fine-scale biocomplexity was found in a species that is widely distributed

and has high potential for dispersal contradicts traditional notions of genetic homogeneity in marine systems.

Most studies of adaptive divergence in the ocean have focused on relatively broad spatial scales (e.g. Conover & Present 1990; Bricelj et al. 2005; Hutchings et al. 2007; Bradbury et al. 2010; Baumann & Conover 2011), but evidence of small-scale adaptive variation in marine species is growing. A recent investigation into the spatial scale of variation in adapted traits in the Atlantic silverside (*Menidia menidia*) revealed significant differences in growth rate, vertebral number, and sex determination at spatial scales of ≈ 60-80 km in addition to broad scale clinal variation, although it is not known whether these fine-scale differences are adaptive (Hice et al. 2012). Perhaps the smallest scale at which potentially adaptive genetic variation has been documented in the ocean is 5 km, between populations of tropical sea anemones (*Condylactis gigantea*; Stoletzki & Schierwater 2005).

Earlier common-garden experiments on cod have revealed genetic differences at relatively broad scales in adaptive traits including larval growth rate (Purchase & Brown 2000) and thermal plasticity for growth rate (Hutchings et al. 2007), food conversion efficiency (Purchase & Brown 2000) and condition factor (Purchase & Brown 2001) in juveniles, and the effect of light intensity on larval growth and survival (Puvanendran & Brown 1998). Differences in body shape plasticity were documented between two winter-spawning components of the 4X division located <100 km apart, although the adaptive significance of these differences is unclear (Marcil 2004; Marcil et al. 2006). Though not from common-garden experiments, there is variation in maturation reaction norms between Skagerrak coastal cod inhabiting neighbouring fjords that is comparable to divergence at neutral genetic markers (Olsen et al. 2008).

The significant genetic variation in reaction norms observed between 3Ps, 4T, and 4X-Bay of Fundy (shown here and in Hutchings et al. 2007) is not matched by differentiation at neutral markers (Hardie et al. 2006). The lack of correspondence between neutral and adaptive markers provides evidence of genetic structure resulting

from selection persisting in the face of apparently high gene flow (Hutchings et al. 2007). With regards to the smallest scale at which adaptive divergence was detected in this study, the degree to which the fall-spawning 4X-Sambro group and the winter-spawning 4X components may intermix is not known. Given the considerable differences in spawning times, and the limited migration and apparently low levels of gene flow between the winter-spawning components (Ruzzante et al. 1998), differentiation of Sambro cod from the remaining 4X spawning components at neutral markers seems likely.

As in Chapter 2, I interpret these differences in plasticity for larval growth and survival to be of genetic, rather than maternal origin. This is supported by a lack of relationship between large sizes at hatch and higher growth or survival for the 4X spawning components. Other potential causes of reaction norm variation in these experiments include size-selective mortality, variation in larval density among tanks, and that experiments were carried out at different times over the course of a decade. If size-selective mortality were responsible for the differences in length observed between groups, we would expect to see changes in length corresponding to changes in survival. This was not the case. For example, there was no difference in length of 4X-Sambro larvae between temperature treatments despite a three-fold difference in survival. I also showed that random effects in the growth model were not related to tank density. A lack of association between lower survival (i.e. lower density) and high growth provides further evidence that variation in density is not responsible for the patterns of phenotypic variation observed. Finally, that the common-garden experiments were conducted at different times may have influenced the mean trait values of the reaction norms. The reaction norm slopes are unlikely to be affected, as demonstrated by a lack of difference in growth plasticity between two groups of 4T cod that were studied eight years apart. However, perhaps with changing ocean temperatures and sufficient time scales for evolution in plasticity to occur, this temporal variation in reaction norm slopes could become apparent.

In summary, I found evidence of adaptive divergence in a widely distributed, broadcast-spawning marine fish at a spatial scale smaller than has been previously observed across open waters in a marine fish. Thermal plasticity for larval growth and survival differed among cod populations located less than 250 km apart. The patterns of plasticity for growth and survival are consistent with the hypotheses that the winter- (4X-Bay of Fundy) and spring- (3L, 3Ps, and 4T) spawning populations are adapted to the seasonal warming experienced during the larval stage and that populations that experience relatively cold temperatures are more sensitive to changes in temperature, whereas the fall-spawning (4X-Sambro) group is adapted to the seasonal cooling experienced during the larval stage and is unable to cope with higher temperatures. This study adds to the growing body of research supporting small-scale local adaptation in the marine environment and the important influence of water temperature on life-history traits in cod and in promoting adaptive divergence among populations.

# Chapter 4: Conclusion

## 4.1 Introduction

My research has demonstrated that genetic divergence in plasticity for adaptive traits exists across a wider range of thermal environments and at a smaller spatial scale than has been previously shown for Atlantic cod. In Chapter 2, I provided compelling evidence of variation in both adaptive and non-adaptive plasticity in two cod populations across a broad range of temperatures that encompassed those typically experienced by both populations in their contrasting native environments. In Chapter 3, I found that variation in adaptive traits occurs at a smaller spatial scale than has been previously documented for a marine fish species in the absence of physical barriers to gene flow. I hypothesized that the fine-scale adaptive divergence observed between two 4X spawning components is likely the result of local adaptations to different seasonal changes in temperature during the larval stage, although behavioural barriers to gene flow, such as variation in spawning times and limited migration, likely play an important role in maintaining these adaptations.

## 4.2 Future Implications

Marine fish species around the world are facing unprecedented threats from direct (e.g. overfishing) and indirect (e.g. climate change) anthropogenic disturbances and their interactions (Hutchings & Reynolds 2004; Mora et al. 2007). Perhaps no other species has felt these impacts more severely than Atlantic cod, which are estimated to have declined by more than 90% in Canadian waters from 1962-1992 (Hutchings & Rangeley 2011) primarily due to overfishing (Hutchings & Myers 1994), although decreasing water temperatures have been cited as a contributing factor (deYoung & Rose 1993). Prevention of further loss of biodiversity and promotion of the recovery of depleted populations will require management strategies that consider both ecological and

evolutionary responses of cod to their ever-changing environments that are based on appropriate spatial scales (Hutchings et al. 2007; Olsen et al. 2008).

### *4.2.1 Potential Impacts Of Climate Change*

Given the high levels of plasticity in life-history traits in some cod populations, even a small, sustained change in ocean temperature could have major impacts on population growth rate and recovery (Drinkwater et al. 2005). A 2-4°C increase in mean temperature, as predicted by climate models to occur by the year 2100 (IPCC 2007), would likely result in faster larval growth for all populations except 4X-Sambro, for which growth would remain unchanged. The increases in growth for the winter- and spring-spawning populations would be complemented by survival rates that are at least constant (3L, 3Ps, and 4T) or increased (4X-Bay of Fundy), though in the wild these survival rates would likely improve with faster growth reducing the risk of predation. Conversely, my findings suggest that 4X-Sambro larvae would experience drastically higher mortality with even slight increases in temperature. Therefore rising ocean temperatures could result in a net increase in productivity for 3L, 3Ps, 4T, and 4X-Bay of Fundy cod but a severe decline in 4X-Sambro cod productivity.

There are a number of scenarios in which increases in glacial meltwater from Greenland may actually lead to periods of cooling in some areas (IPCC 2007). In the event that temperatures decline, only 4X-Sambo cod may benefit from increased productivity while the remaining populations would be at risk of lower growth rates and declining larval survival. Though survival of 4X-Sambro cod is likely bounded by near-freezing temperatures, so the benefits would be limited.

These predictions assume a homogeneous change in temperature on a large spatial scale and ignore other changes to the ecosystem that could result from climate change such as range shifts and changes in food availability (reviewed in Pörtner & Peck 2010). For example, cod populations could adjust their spawning times or locations in response to climate change. However, whether these strategies are effective will depend on how

well they are matched by those of other species in the ecosystem (e.g. prey species; Stenseth & Mysterud 2002), which will depend on the thermal tolerance of each species (Pörtner & Peck 2010). I also assumed that populations would exhibit the same responses in the wild as they did in the lab, which is unlikely as food availability and predation are major factors affecting larval growth and survival in the wild. Nonetheless, the thermal responses described in the present study provide the basis for more elaborate predictions. A more detailed discussion of the impacts of climate change on Atlantic cod that considers numerous variables is available (Drinkwater et al. 2005), but their analysis does not include the 4X-Sambro cod.

The long-term (i.e. evolutionary) consequences of climate change on cod populations will depend on the amount of heritable variation in thermal reaction norms they possess. Variation in adaptive plasticity at the population level increases the likelihood of at least one population having a response that is adaptive in the new environment. The variety of responses observed in this study alone would suggest at least one cod population would be well suited to any (small) directional change in temperature. Selection can also act on variation contained within populations to shape a norm of reaction that is adaptive to future thermal environments (Ghalambor et al. 2007). Future research should seek to quantify the variation in plasticity that exists within populations (e.g. at the family level) in order to assess the adaptive potential of individual populations.

### *4.2.2 Management Implications*

From a management perspective, the maximum potential for evolutionary success is achieved by preserving the maximum diversity of adapted genes across the range of a species (Lande & Shannon 1996; Crandall et al. 2000). Not only can excessive fishing pressure exacerbate the negative consequences of environmental change but it can also cause reductions in genetic diversity that diminish the adaptive potential of a population or species. Therefore managers should prioritize the conservation of adaptive diversity to promote the greatest possible resilience in a species in the face of unpredictable

environmental change (Hutchings & Rangeley 2011). This can be achieved by managing fisheries at a spatial scale that corresponds to the spatial scale of genetic variation (Hutchings et a. 2007). Not accounting for such intraspecific genetic diversity is likely to have negative impacts on fisheries (Hilborn et al. 2003; Ruzzante et al. 2006). For example, management of stocks on a broad scale can lead to the extinction of subpopulations and reduce the overall productivity and resiliency of the stock (Frank & Brickman 2000). The finding that cod are likely to be locally adapted to their environments at both small and broad scales further complicates management because it means that depleted populations may not be readily replaced by neighbouring populations. Therefore the results of this study suggest that management should occur at a maximum scale of NAFO division as well as at the scale of spawning component when these groups spawn at different times of year. This approach will help ensure that the adaptive diversity contained in unique spawning components is preserved and that cod have the best genetic tools to cope with their changing environment.

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# Appendix A: Temperatures Experienced By Study Populations During the Larval Stage

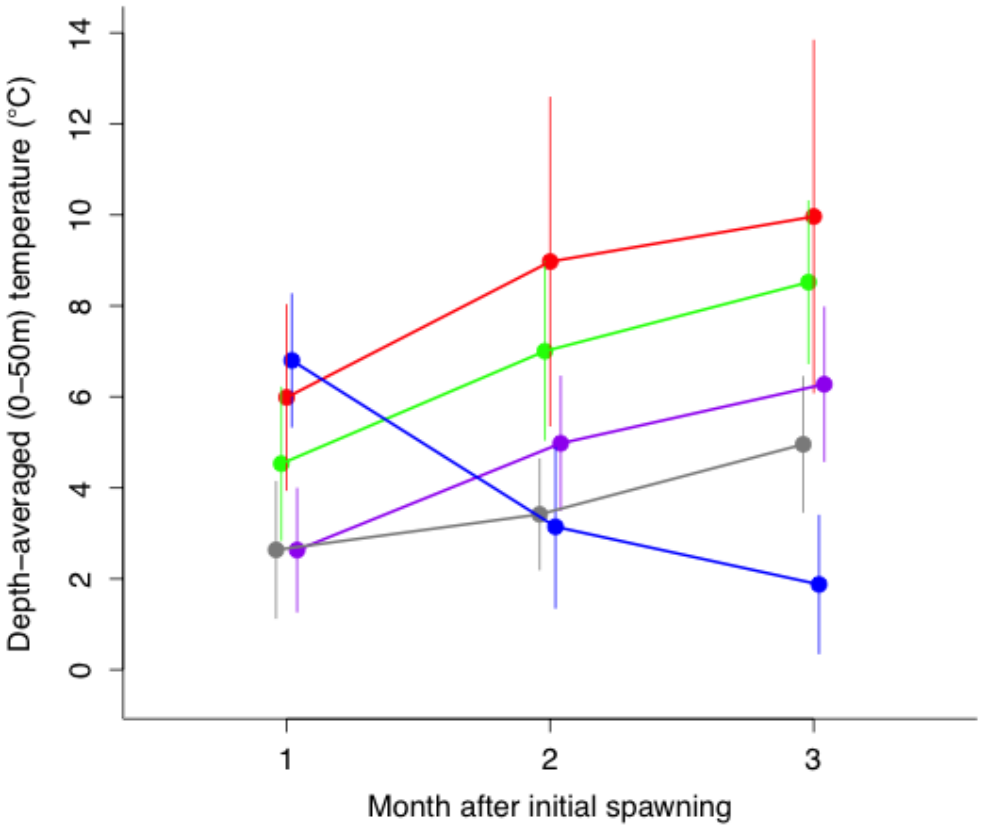


Figure A1: Depth-averaged (0-50 m) water temperatures (°C ±1 SD) experienced by Atlantic cod larvae from 4X-Bay of Fundy (grey), 4X-Sambro (blue), 4T (red), 3L (purple) and 3Ps (green) during the first three months after their initial spawning months (May [4T, 3L, and 3Ps]; February [4X-Bay of Fundy]; November [4X-Sambro]). Mean temperatures were calculated using all available data from 1914-2009 in the Bedford Institute of Oceanography's Hydrographic Climate Database (http://www.bio.gc.ca/science/data-donnees/base/climate-climat-eng.php). The following hydrographic subareas were used: 17-26, 29, 43-44, and 53-55 for 4X-Bay of Fundy; 10-21 for 4X-Sambro; 11-18 for 4T; 29-32, 47-48, and 50 for 3L; 49, 55-56, 58-63, and 65 for 3Ps.

# Appendix B: Model Residual Plots For Section 2.1.4 – Growth Reaction Norms

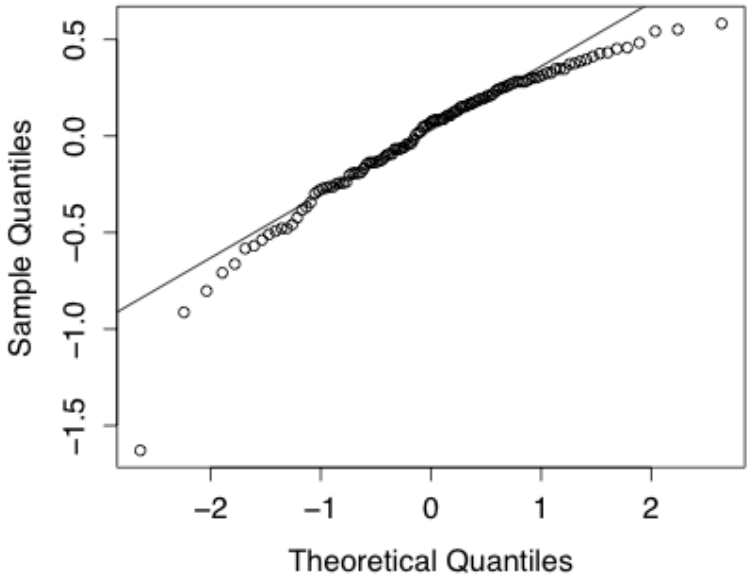


Figure B1: Normal quantile-quantile plot of residuals from model of larval length at hatch.

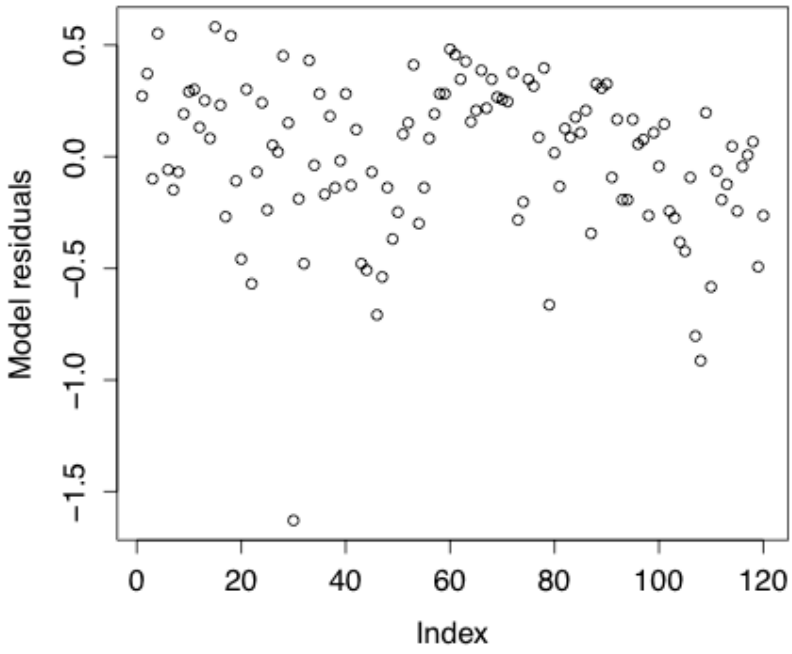


Figure B2: Plot of model residuals for larval length at hatch.

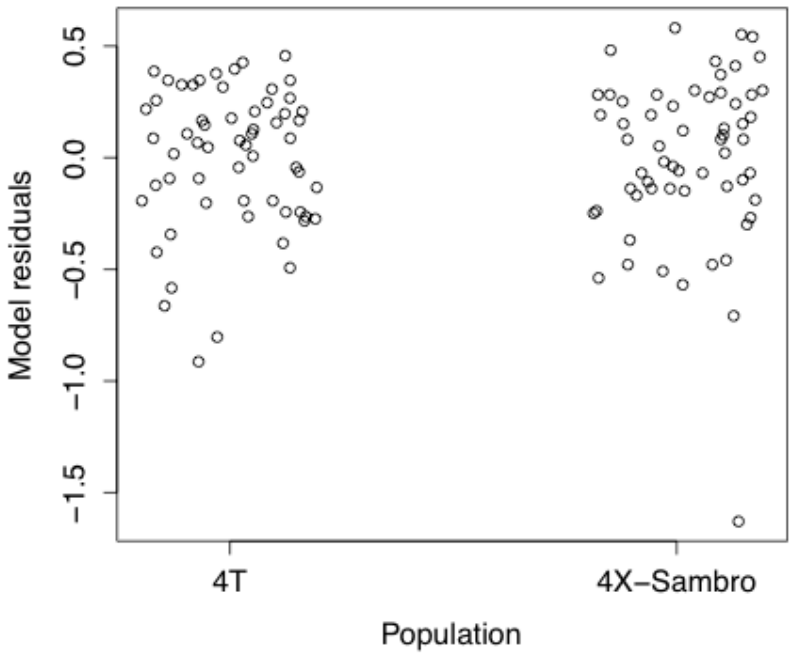


Figure B3: Plot of model residuals by population for larval length at hatch.

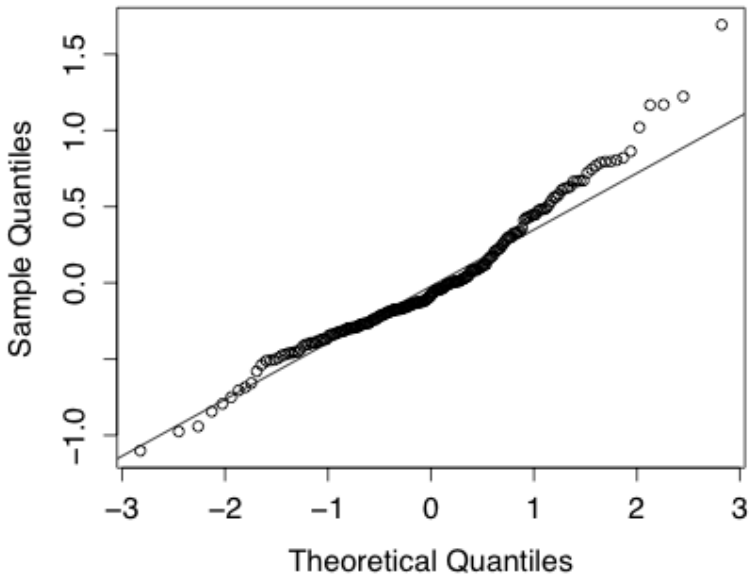


Figure B4: Normal quantile-quantile plot of residuals from model of larval length at day 14.

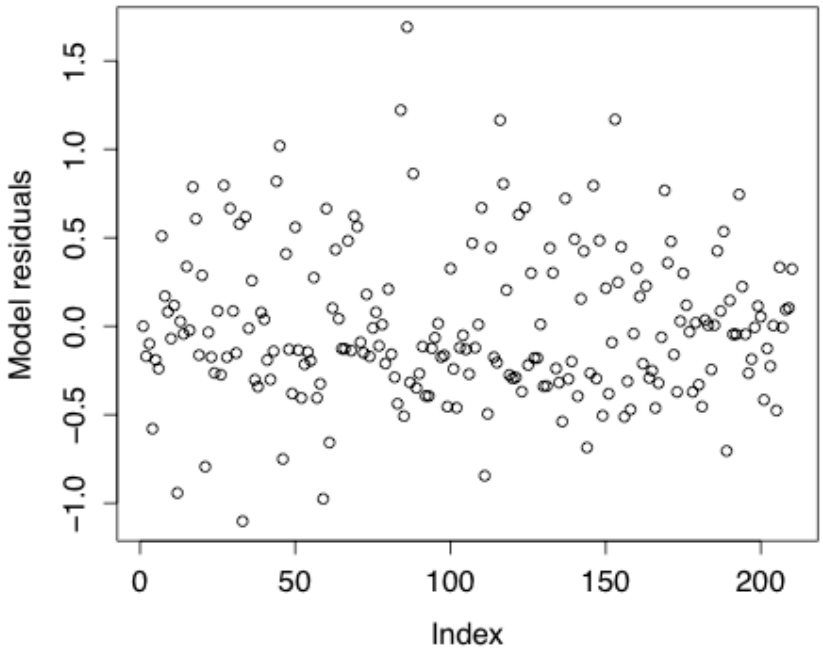


Figure B5: Plot of model residuals for larval length at day 14.

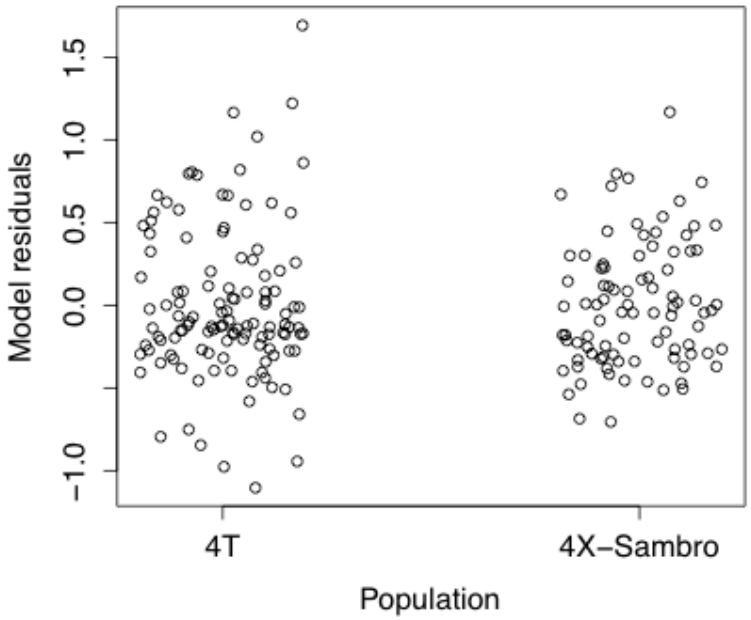


Figure B6: Plot of model residuals by population for larval length at day 14.

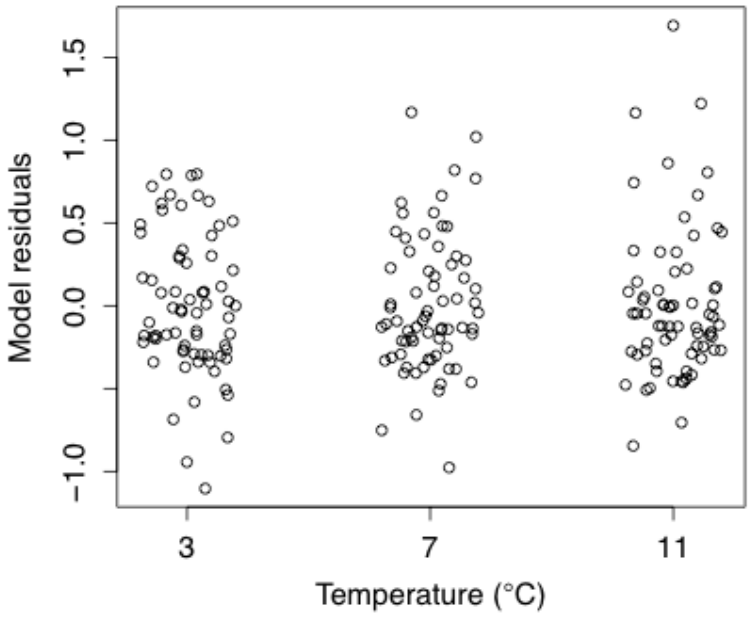


Figure B7: Plot of model residuals by temperature for larval length at day 14.

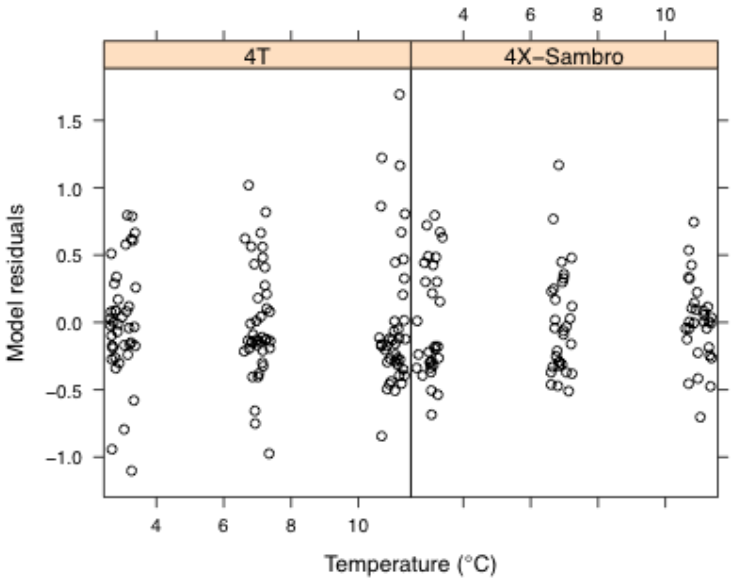


Figure B8: Plot of model residuals by population and temperature for larval length at day 14.

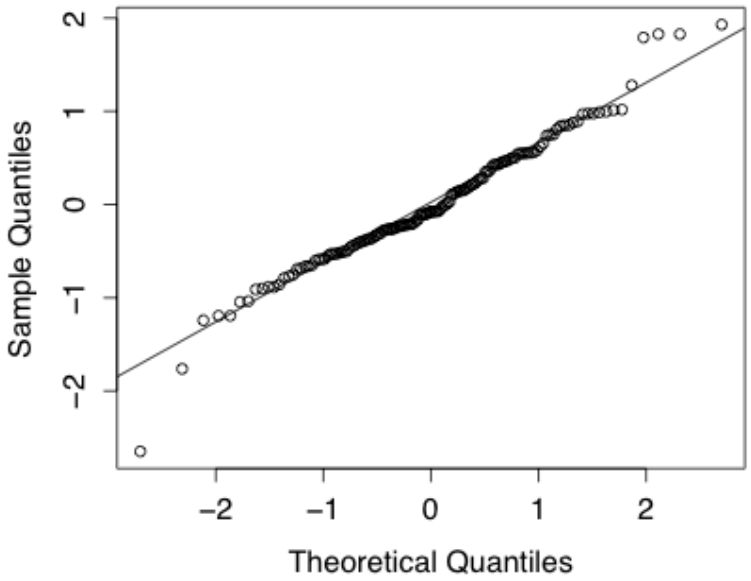


Figure B9: Normal quantile-quantile plot of residuals from model of larval length at day 29.

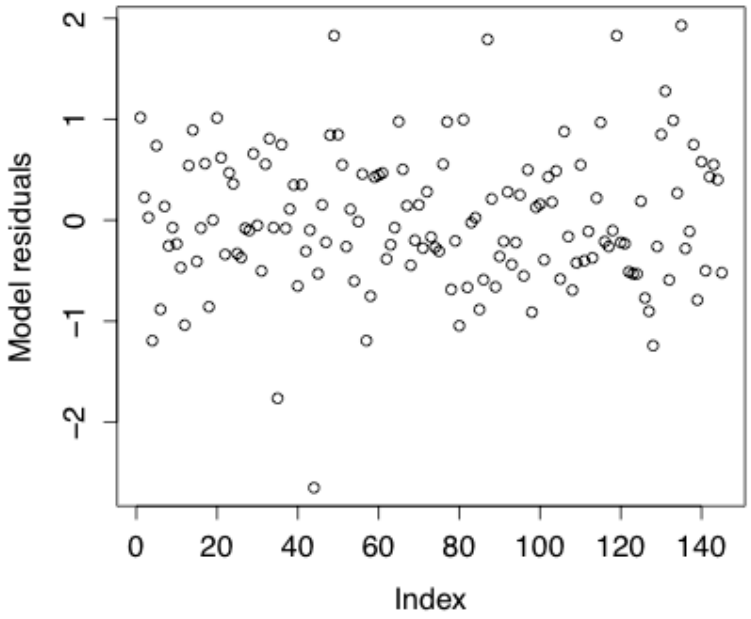


Figure B10: Plot of model residuals for larval length at day 29.

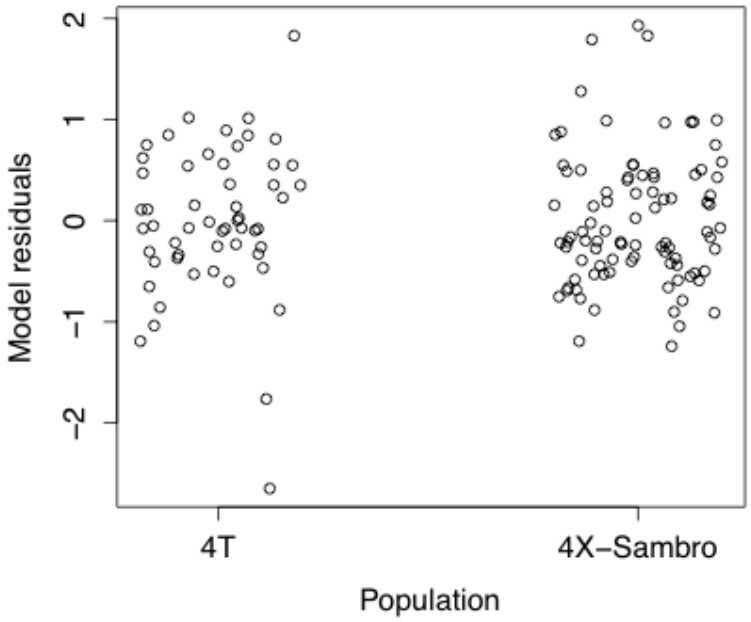


Figure B11: Plot of model residuals by population for larval length at day 29.

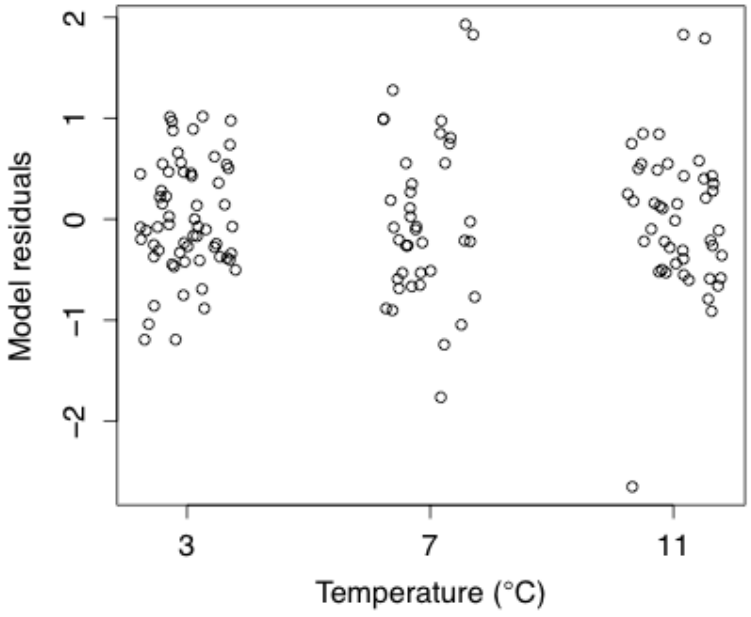


Figure B12: Plot of model residuals by temperature for larval length at day 29.

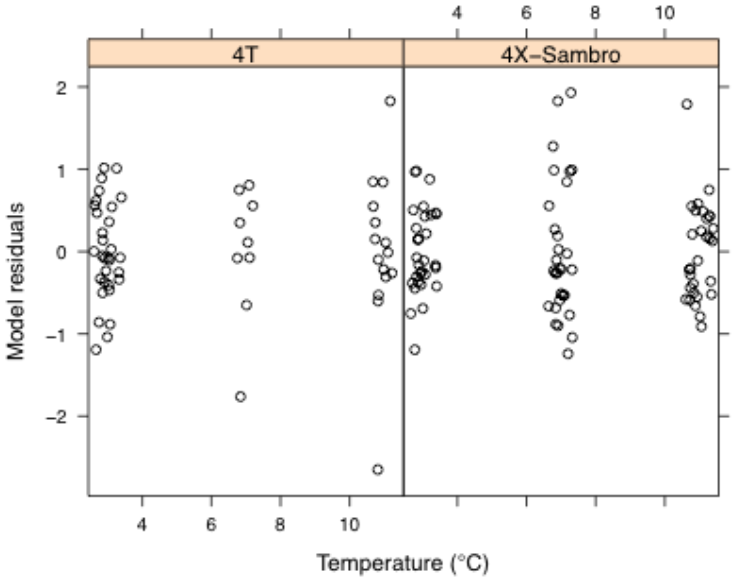


Figure B13: Plot of model residuals by population and temperature for larval length at day 29.

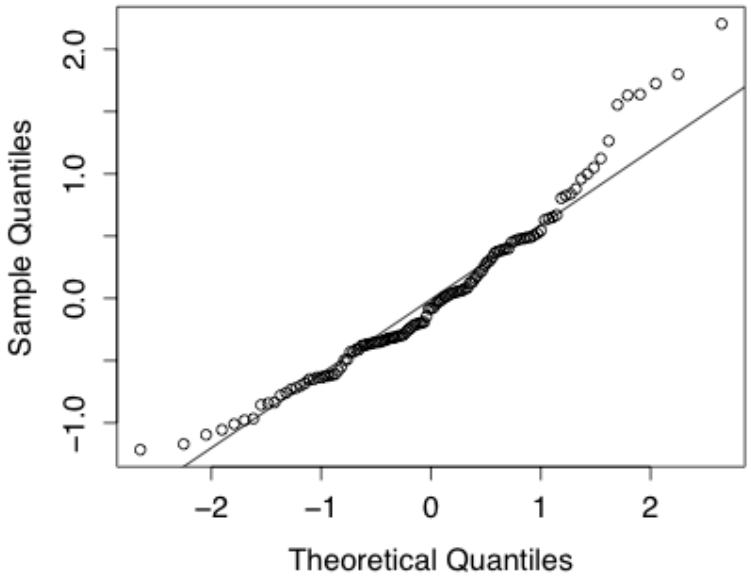


Figure B14: Normal quantile-quantile plot of residuals from model of larval length at day 29 for 4X-Sambro as determined using all available data and using lengths that were averaged within families within temperatures.

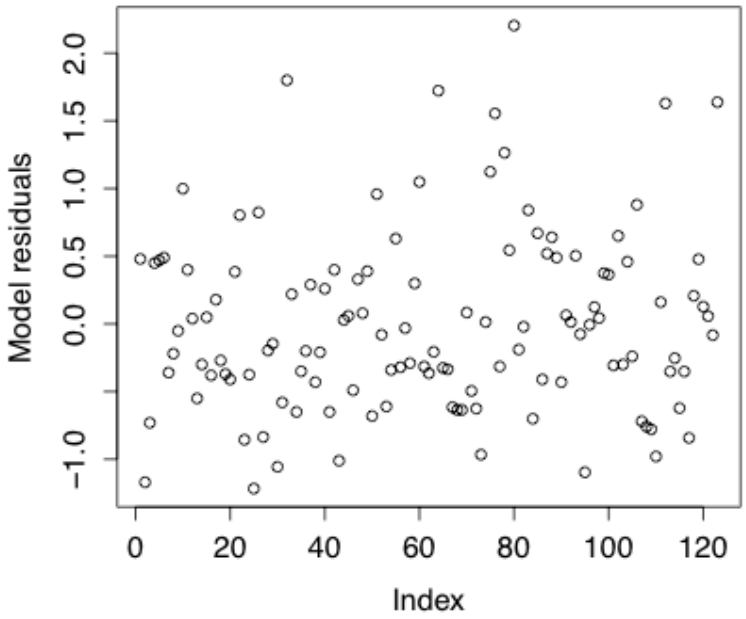


Figure B15: Plot of model residuals for larval length at day 29 for 4X-Sambro as determined using all available data and using lengths that were averaged within families within temperatures.

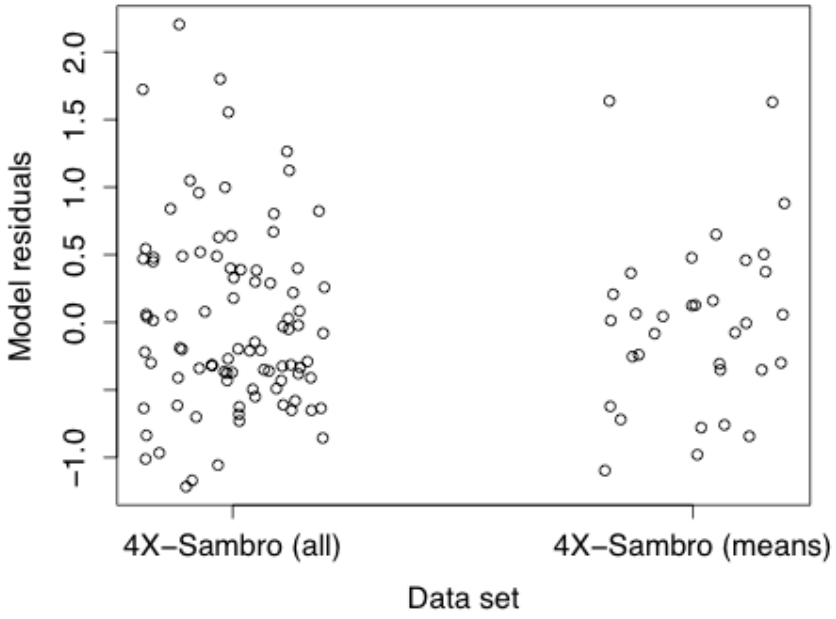


Figure B16: Plot of model residuals by population for larval length at day 29 for 4X-Sambro as determined using all available data and using lengths that were averaged within families within temperatures.

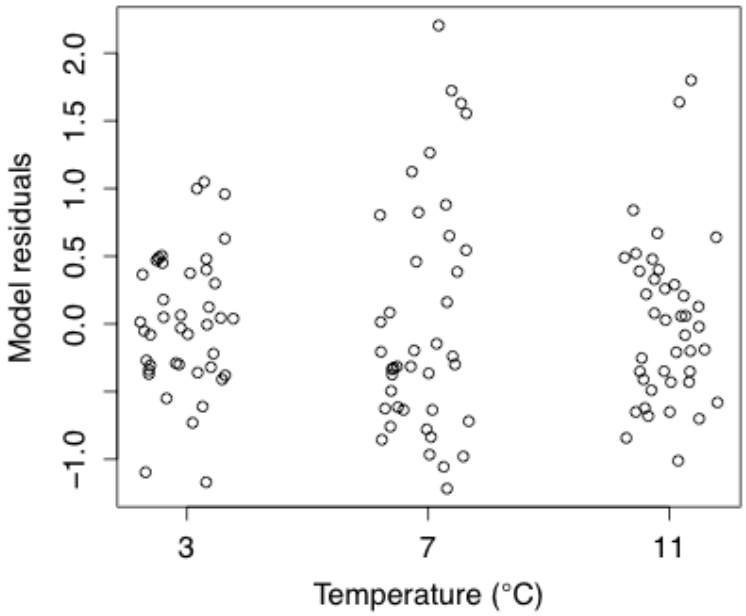


Figure B17: Plot of model residuals by temperature for larval length at day 29 for 4X-Sambro as determined using all available data and using lengths that were averaged within families within temperatures.

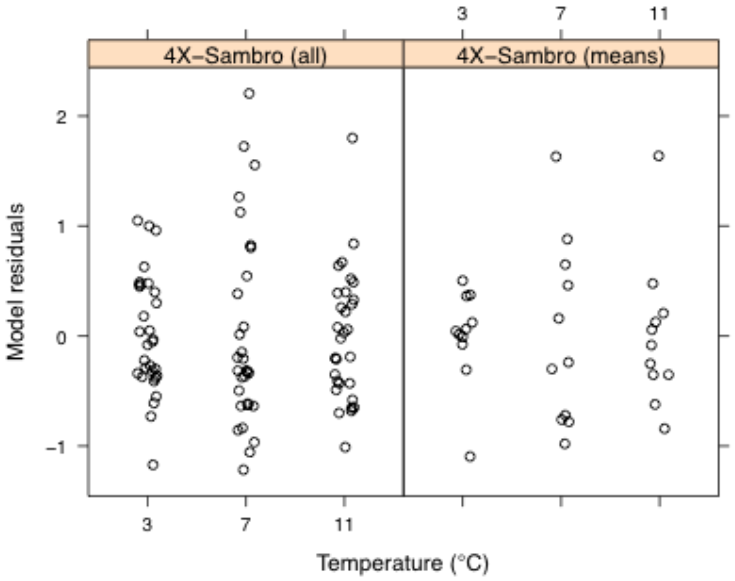


Figure B18: Plot of model residuals by population and temperature for larval length at day 29 for 4X-Sambro as determined using all available data and using lengths that were averaged within families within temperatures.

# Appendix C: Model Residual Plots For Section 2.1.5 – Survival Reaction Norms

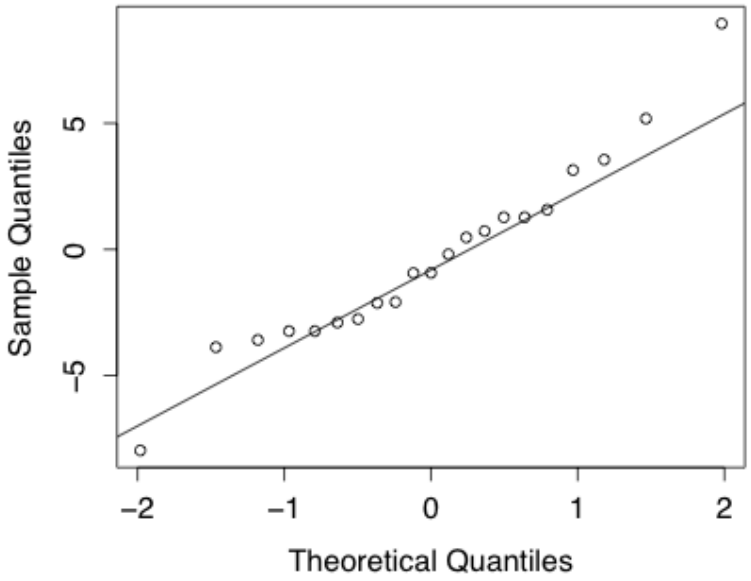


Figure C1: Normal quantile-quantile plot of residuals from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and logit link.

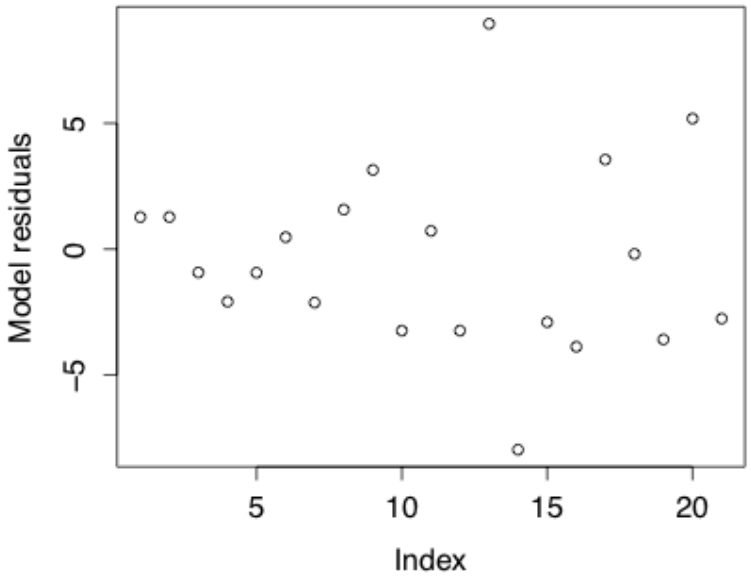


Figure C2: Plot of model residuals from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and logit link.

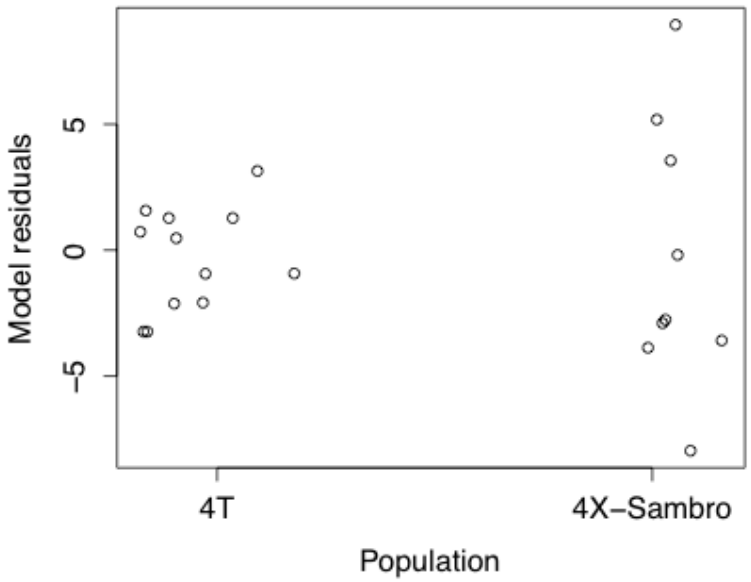


Figure C3: Plot of model residuals by population from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and logit link.

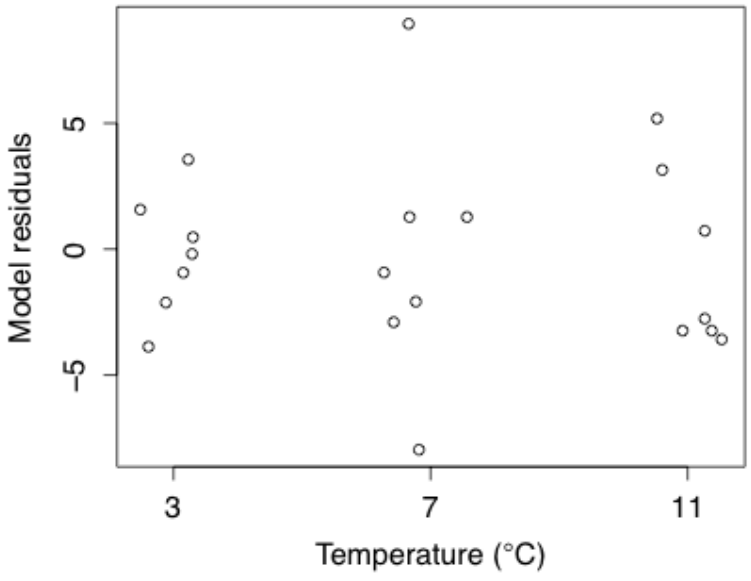


Figure C4: Plot of model residuals by temperature from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and logit link.

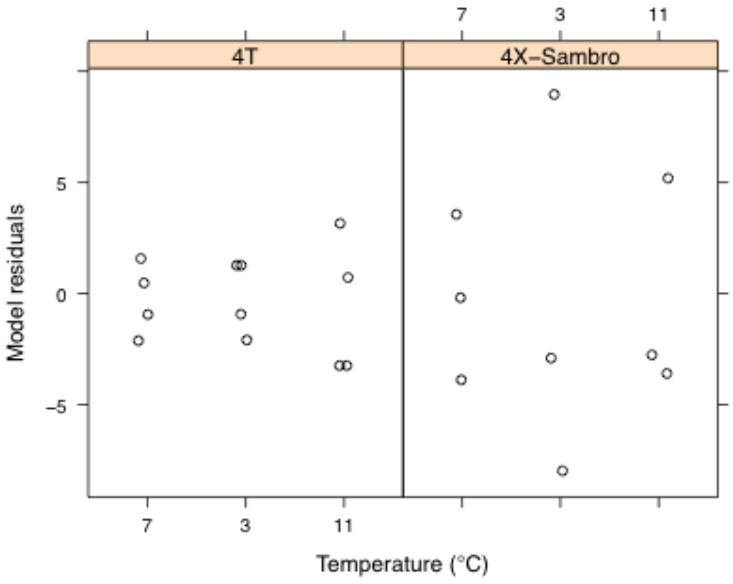


Figure C5: Plot of model residuals by population and temperature from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and logit link.

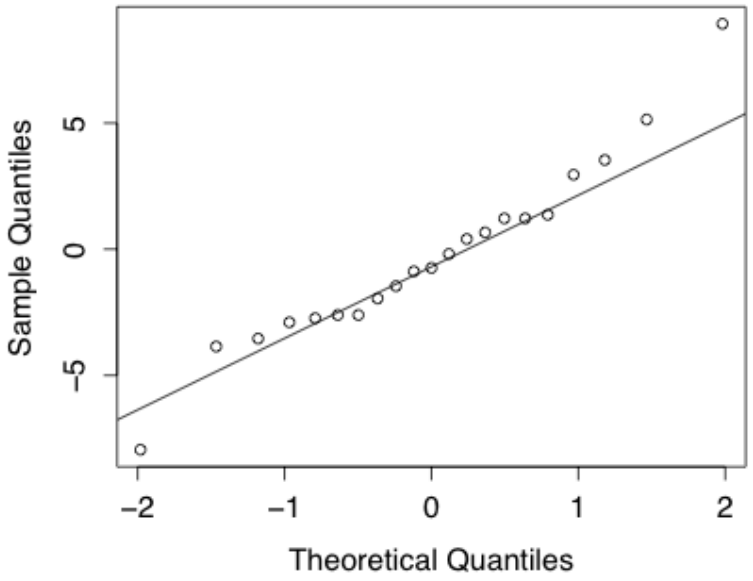


Figure C6: Normal quantile-quantile plot of residuals from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and identity link.

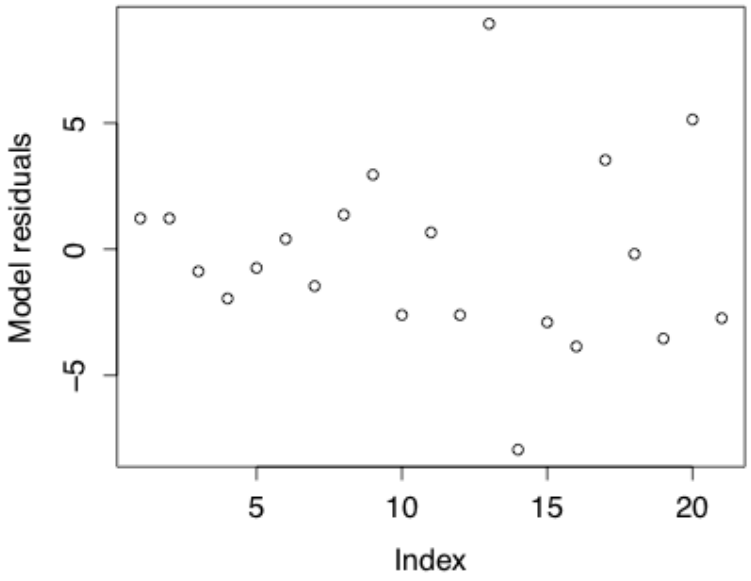


Figure C7: Plot of model residuals from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and identity link.

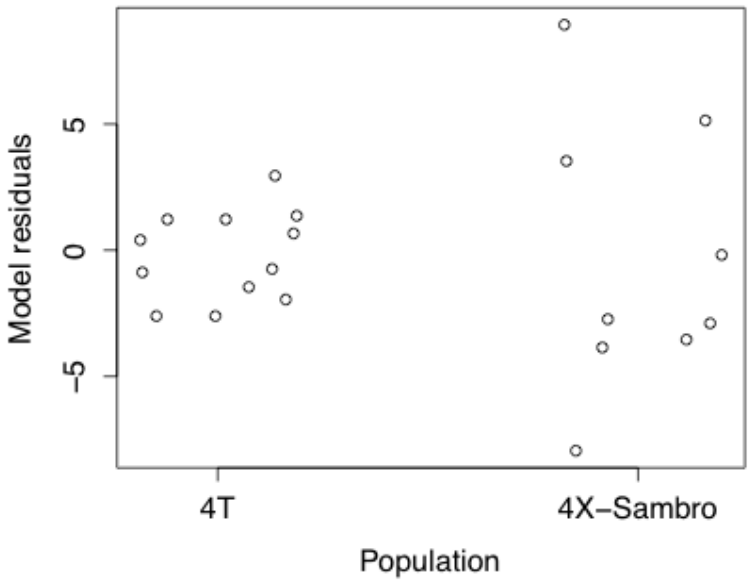


Figure C8: Plot of model residuals by population from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and identity link.

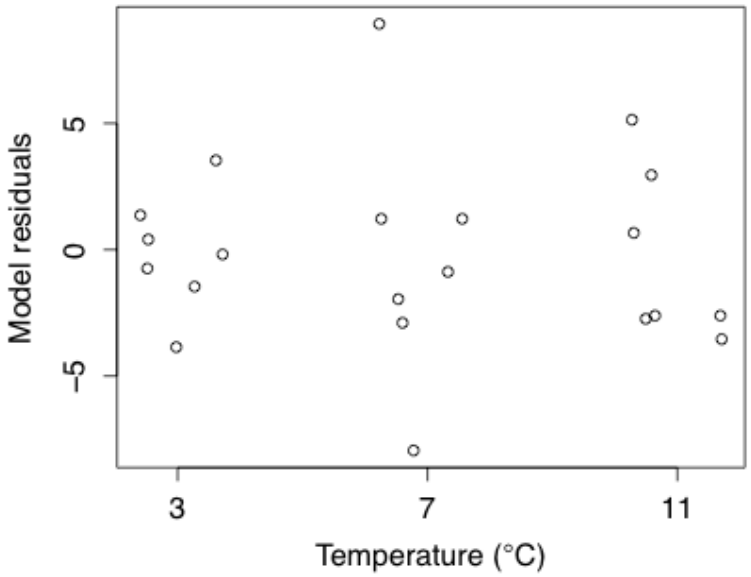


Figure C9: Plot of model residuals by temperature from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and identity link.

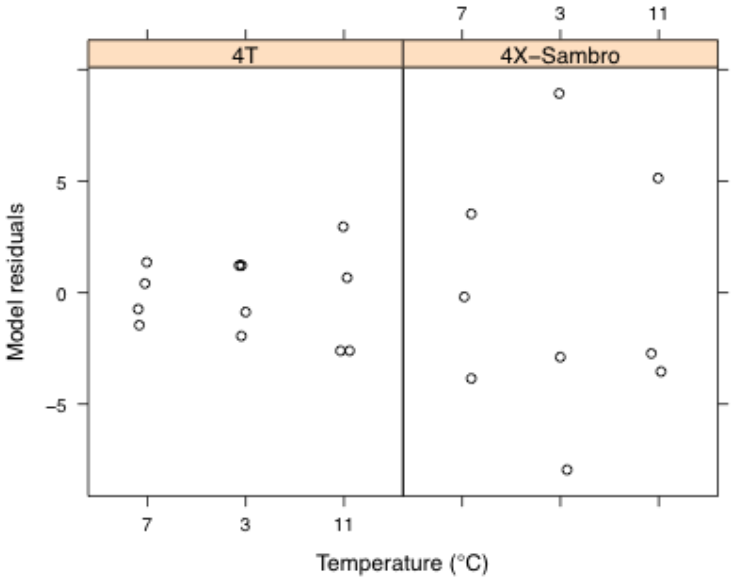


Figure C10: Plot of model residuals by population and temperature from a generalized linear model of larval survival at day 29 using the quasi-binomial distribution and identity link.

# Appendix D: Model Residual Plots For Section 3.1.4 – Growth Reaction Norms

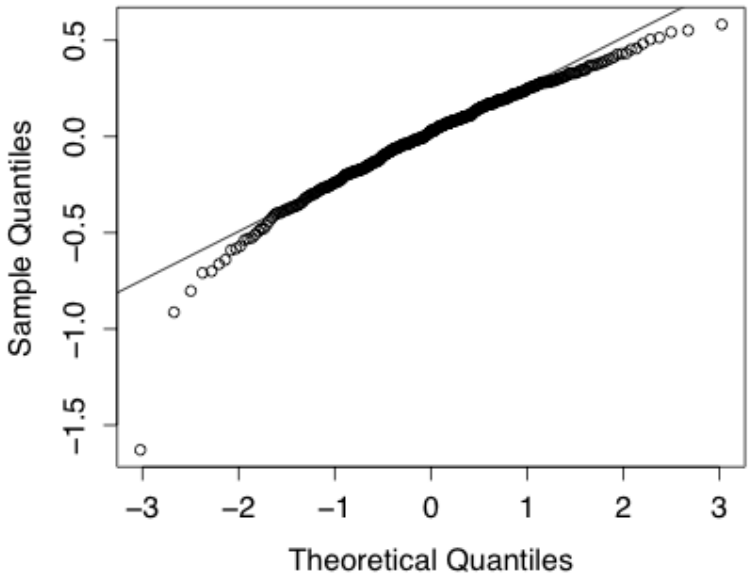


Figure D1: Normal quantile-quantile plot of residuals from model of larval length at hatch.

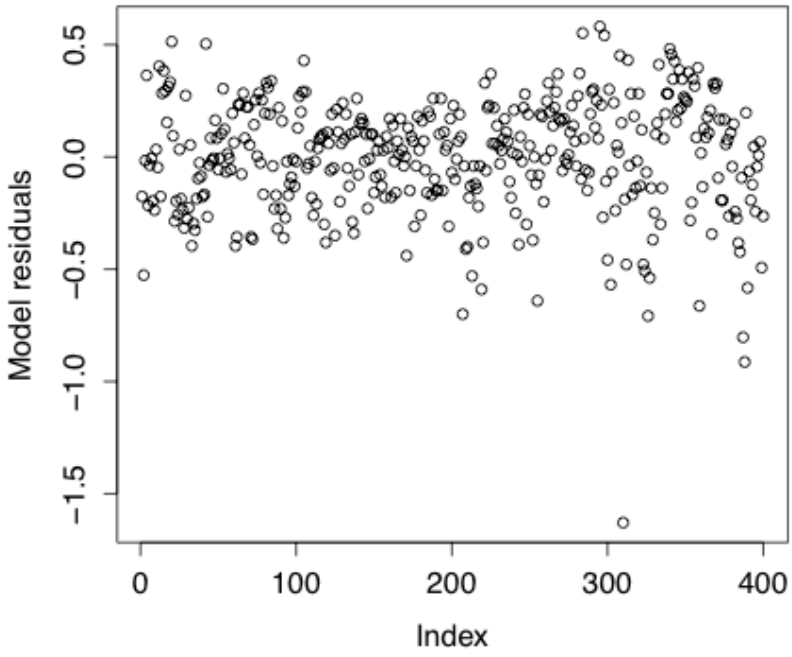


Figure D2: Plot of model residuals for larval length at hatch.

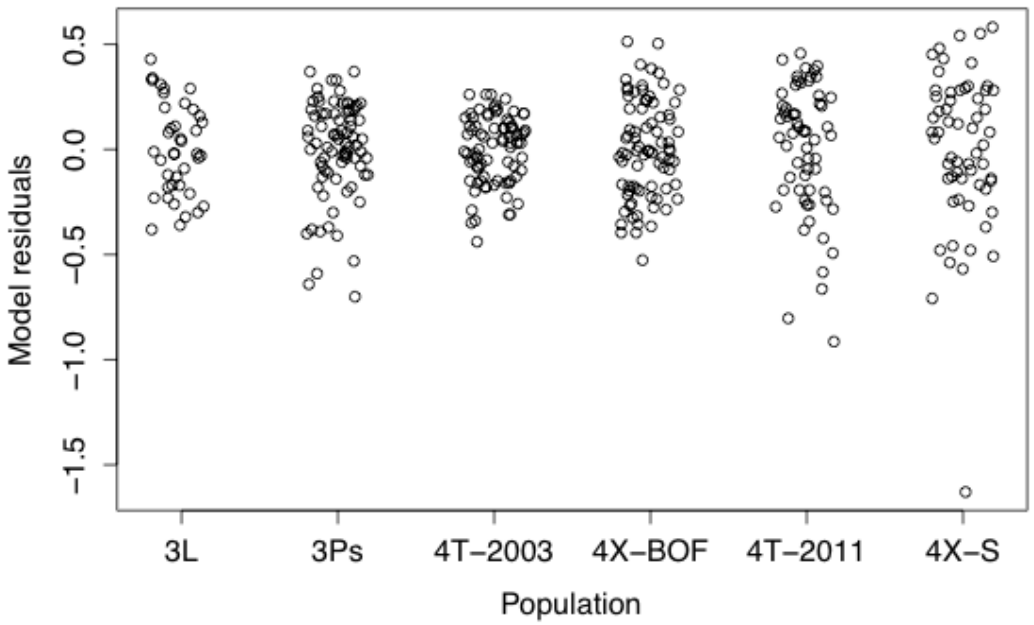


Figure D3: Plot of model residuals by population for larval length at hatch.

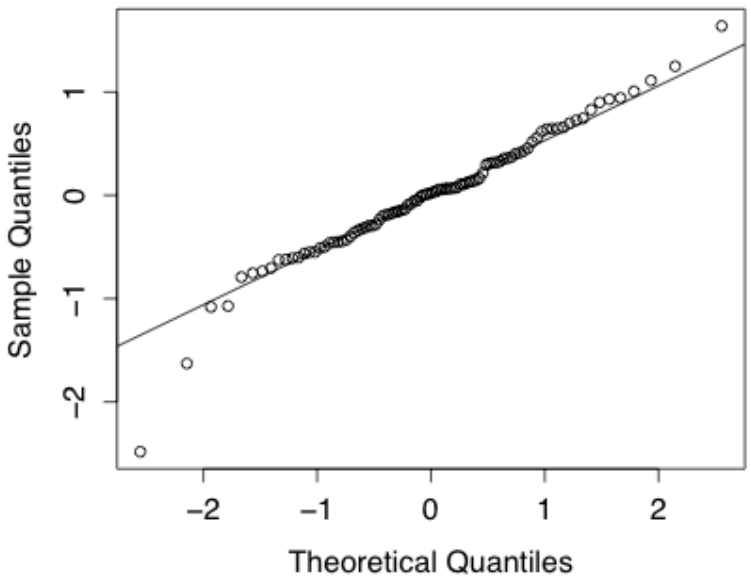


Figure D4: Normal quantile-quantile plot of residuals from model of larval length at day 29 for 4T-2003 and 4T-2011.

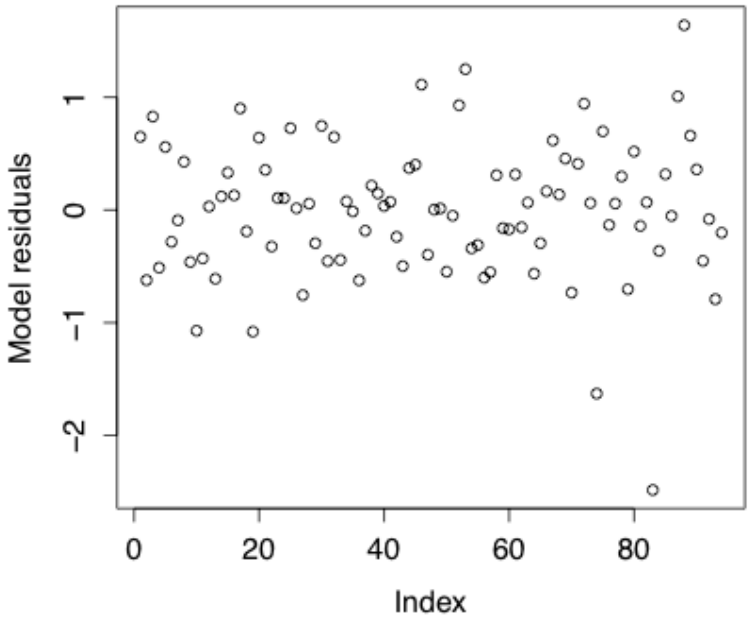


Figure D5: Plot of model residuals for larval length at day 29 for 4T-2003 and 4T-2011.

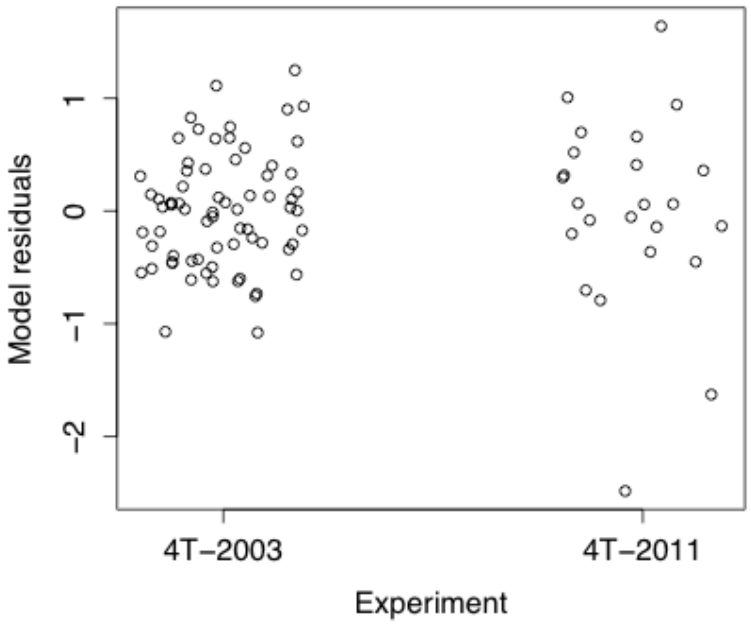


Figure D6: Plot of model residuals by experiment for larval length at day 29 for 4T-2003 and 4T-2011.

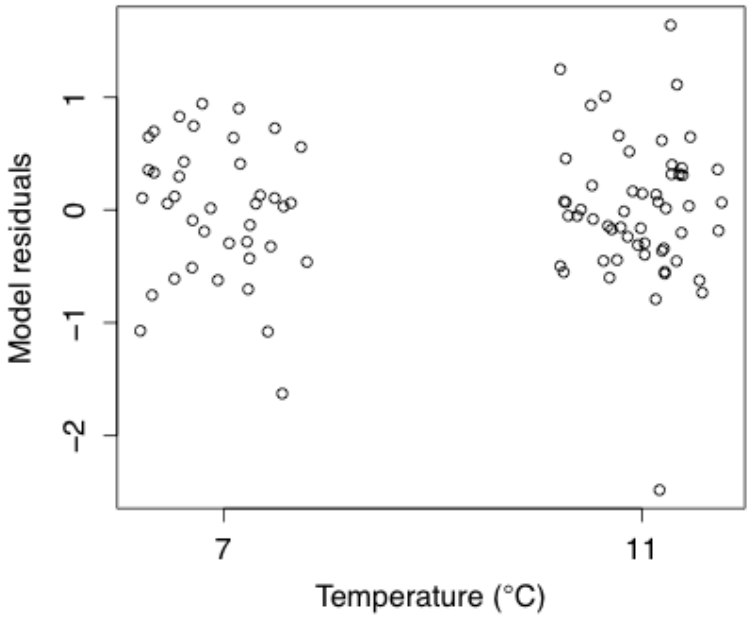


Figure D7: Plot of model residuals by temperature for larval length at day 29 for 4T-2003 and 4T-2011.

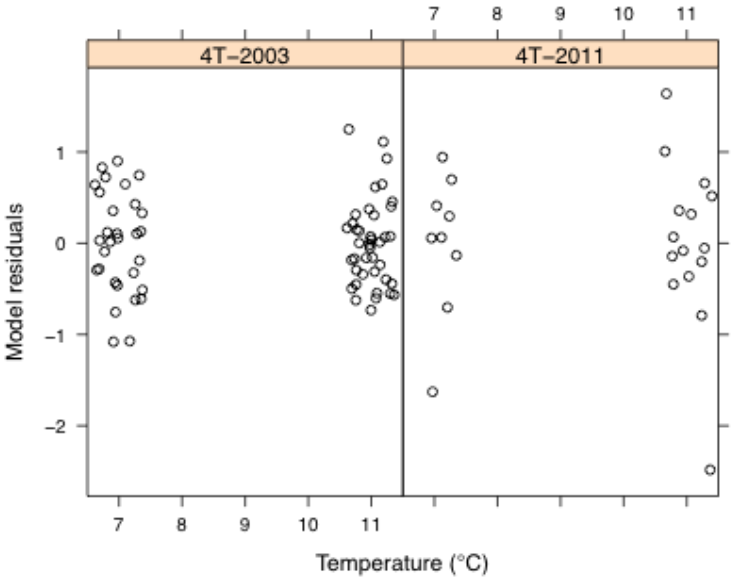


Figure D8: Plot of model residuals by experiment and temperature for larval length at day 29 for 4T-2003 and 4T-2011.

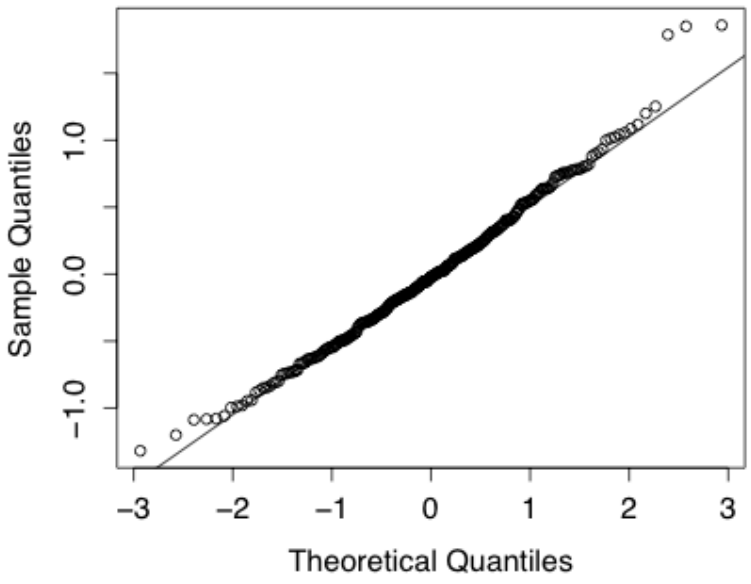


Figure D9: Normal quantile-quantile plot of residuals from model of larval length at day 29.

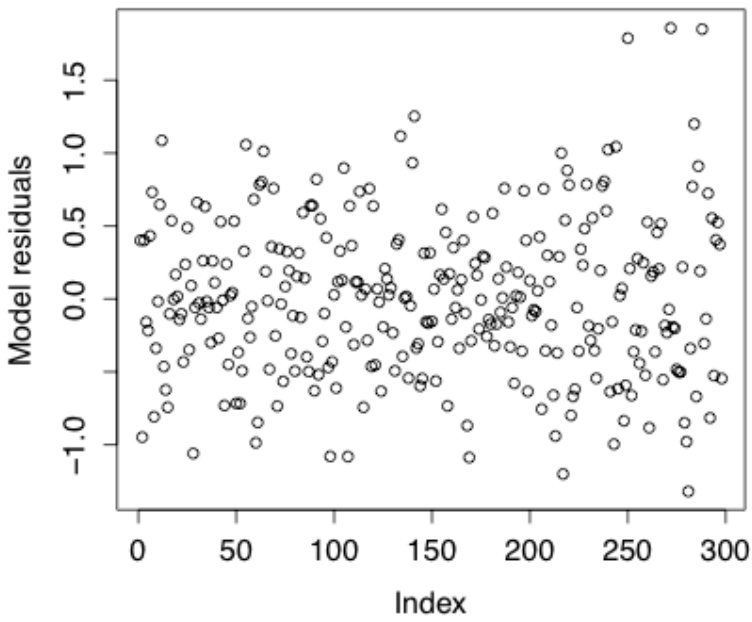


Figure D10: Plot of model residuals for larval length at day 29.

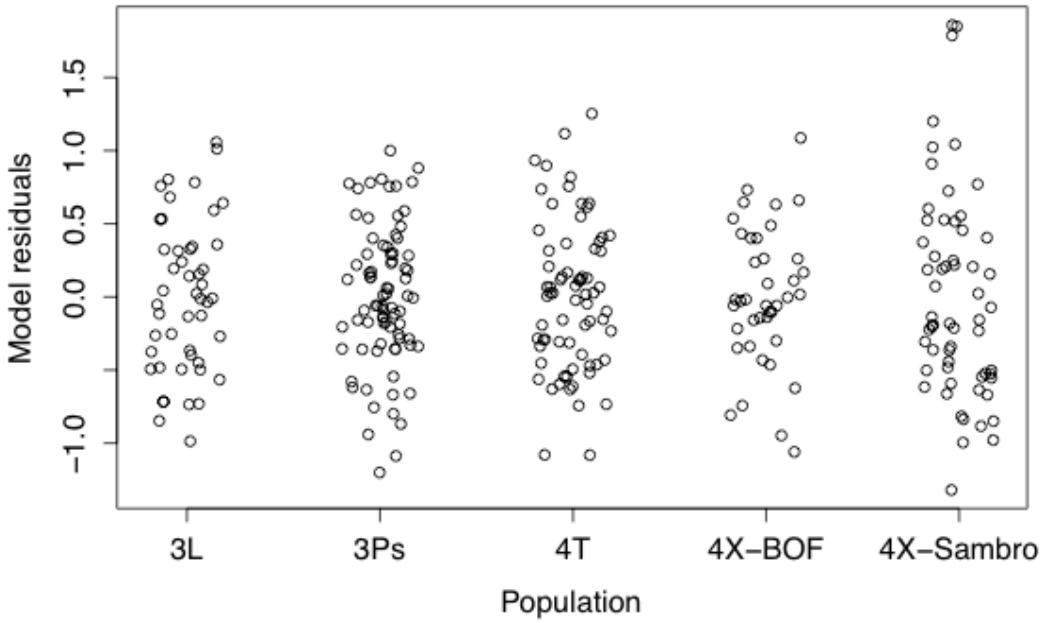


Figure D11: Plot of model residuals by population for larval length at day 29.

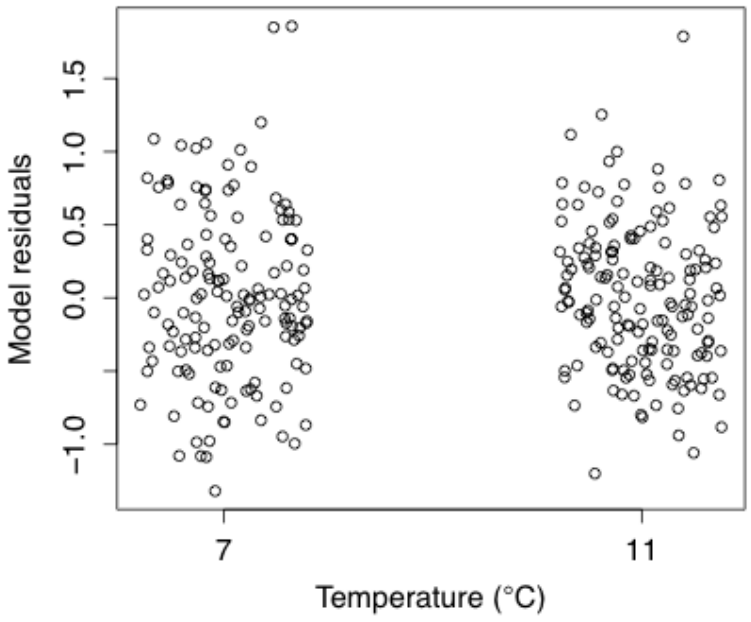


Figure D12: Plot of model residuals by temperature for larval length at day 29.

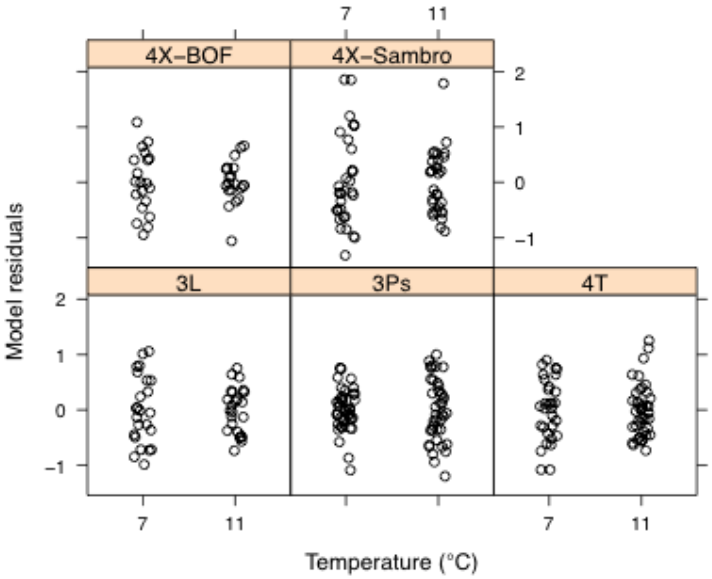


Figure D13: Plot of model residuals by population and temperature for larval length at day 29.

# Appendix E: Model Residual Plots For Section 3.1.5 – Survival Reaction Norms

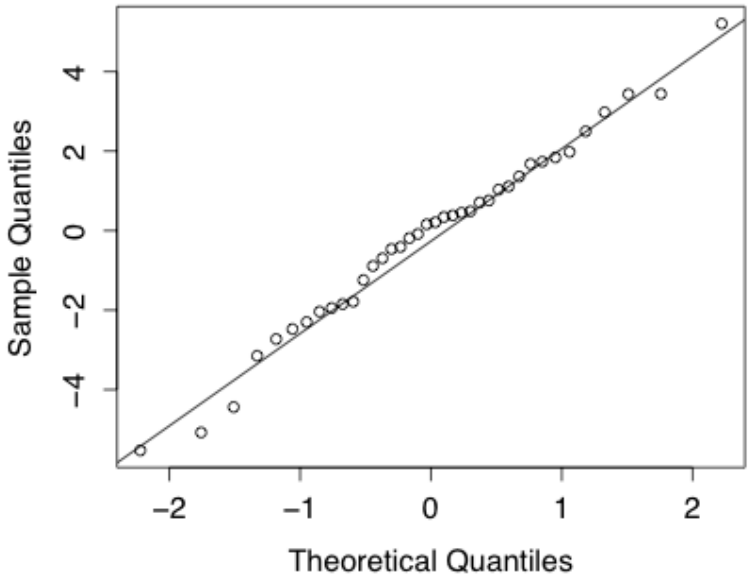


Figure E1: Normal quantile-quantile plot of residuals from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and logit link.

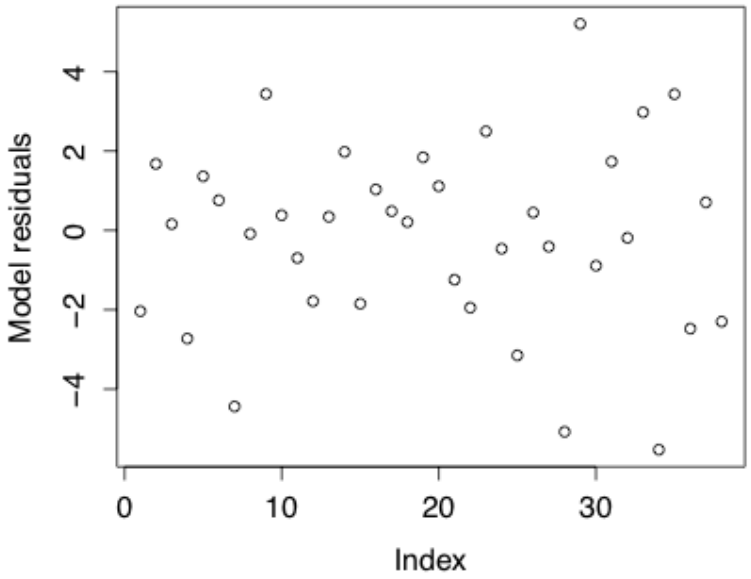


Figure E2: Plot of model residuals from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and logit link.

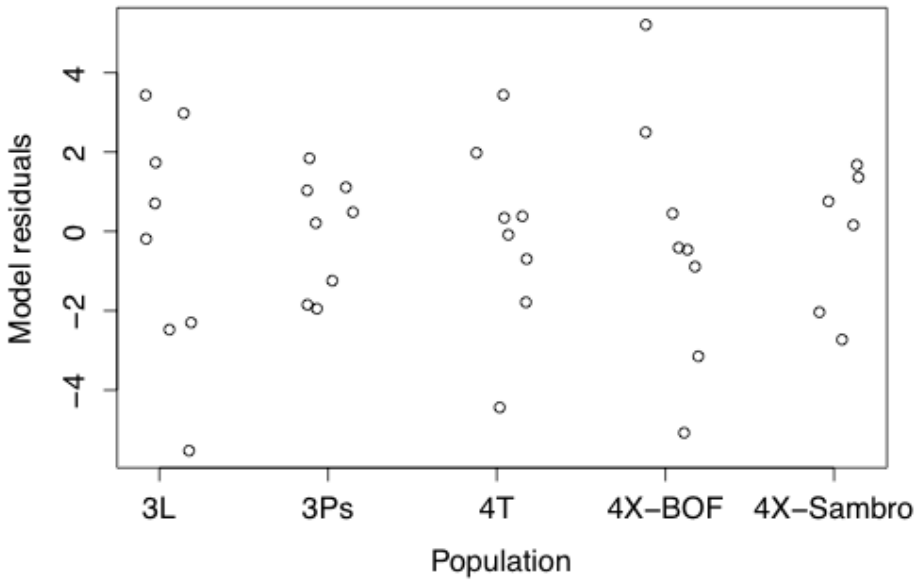


Figure E3: Plot of model residuals by population from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and logit link.

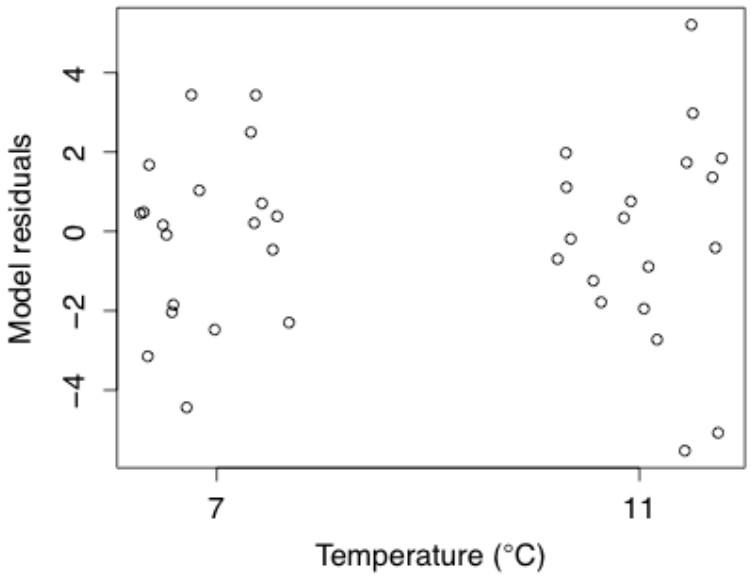


Figure E4: Plot of model residuals by temperature from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and logit link.

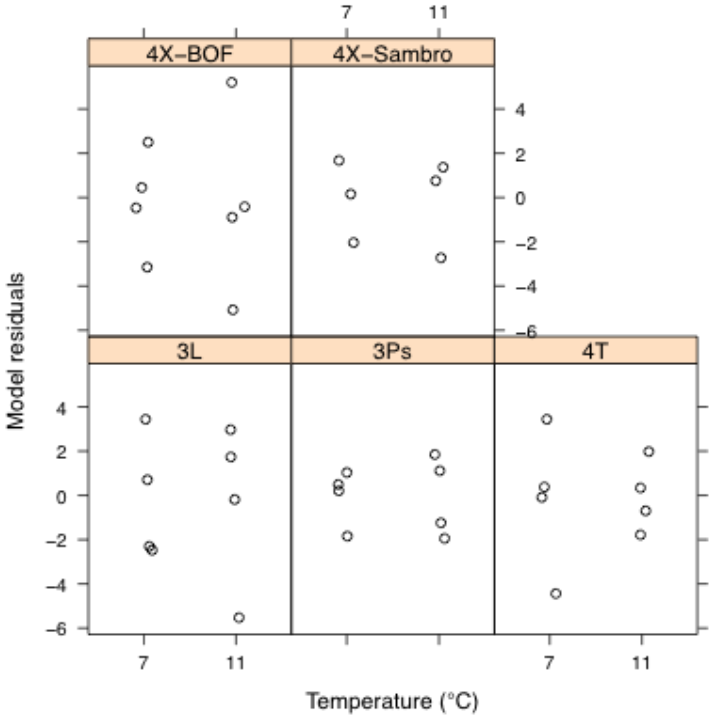


Figure E5: Plot of model residuals by population and temperature from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and logit link.

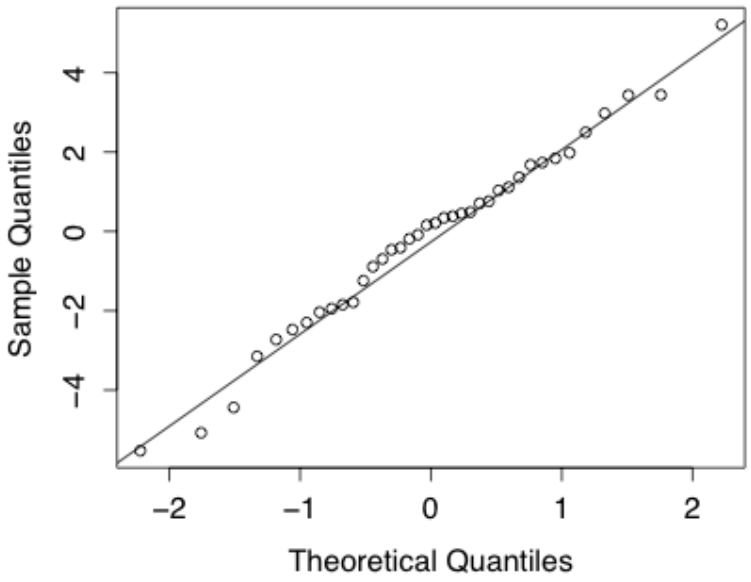


Figure E6: Normal quantile-quantile plot of residuals from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and identity link.

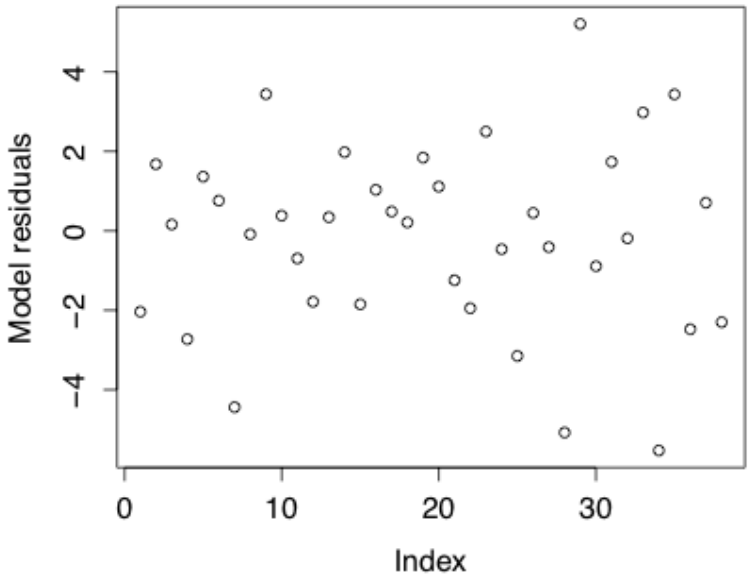


Figure E7: Plot of model residuals from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and identity link.

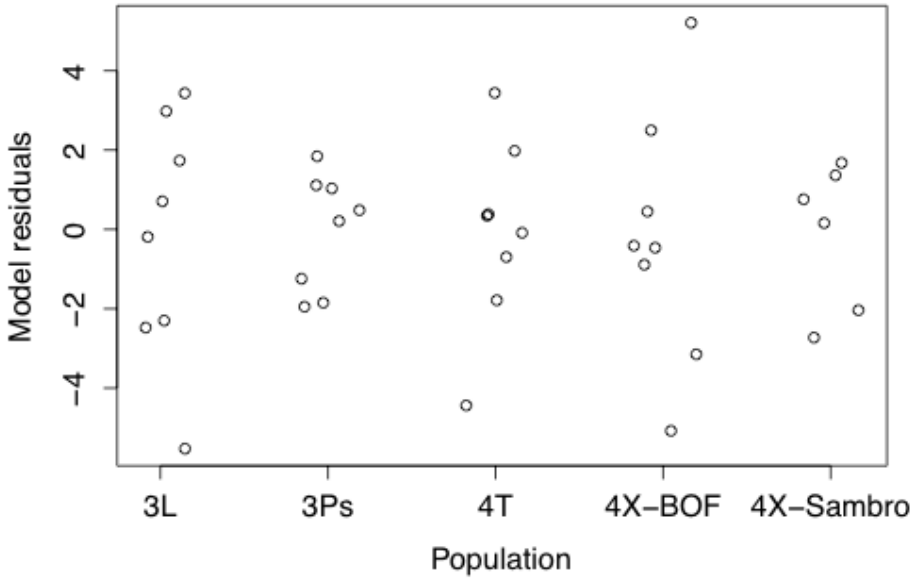


Figure E8: Plot of model residuals by population from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and identity link.

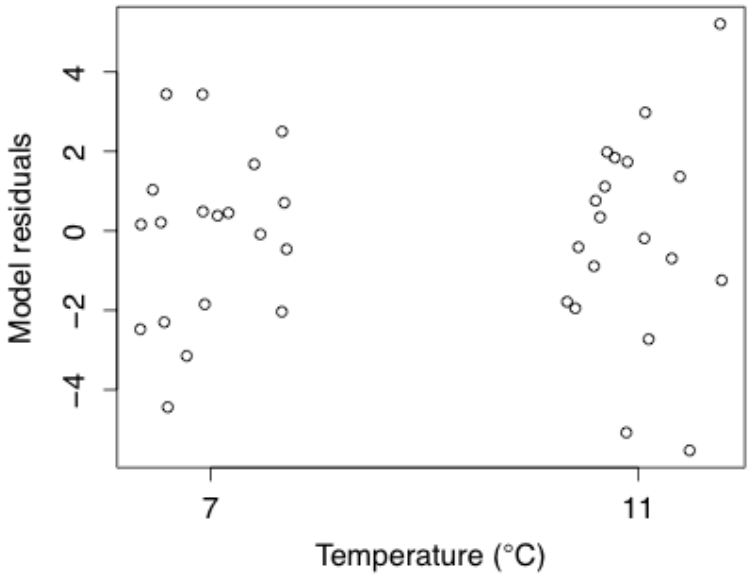


Figure E9: Plot of model residuals by temperature from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and identity link.

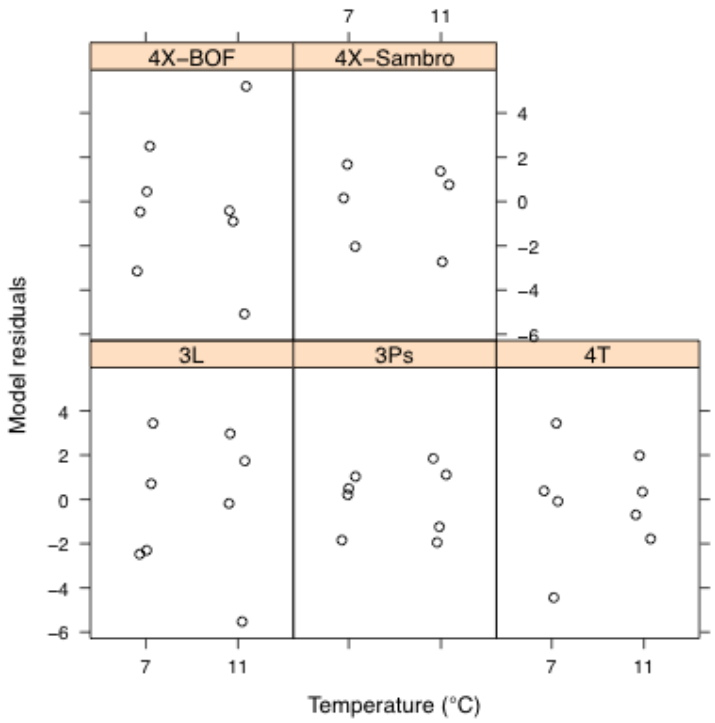


Figure E10: Plot of model residuals by population and temperature from a generalized linear model of larval survival at day 43 using the quasi-binomial distribution and identity link.