Assessing Local Adaptation in Swedish Crayfish

Using Common-Garden Experiments on Life History Traits

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Swedish University of Agricultural Sciences, SLU
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Undersökning av lokal anpassning hos svenska kräftor: Common-garden-experiment på livshistorieegenskaper

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Abstract

Local adaptation is a central concept in evolutionary ecology, and comparative studies of ecologically similar native and invasive species can be used to examine the temporal dynamics of adaptive processes. This thesis investigates these dynamics across native noble crayfish (*Astacus astacus*) and invasive signal crayfish (*Pacifastacus leniusculus*) populations in Sweden. Noble crayfish have occupied diverse climatic regions across Sweden since the last glaciation, which suggests population-specific adaptations in growth and hatching phenology as observed in other ectotherms studied along latitudinal clines. In contrast, signal crayfish are characterised by a shorter colonization history, and certain populations now exhibit declining body sizes, raising questions about whether these trends result from local adaptation or environmentally induced plasticity.

Using common-garden experiments and a P_{ST}-F_{ST} framework, this thesis examines whether population differentiation in both species have a genetic basis. In noble crayfish, population-level differences in growth, hatching date and survival were detected. Local adaptation in growth represents the most probable inference, though P_{ST} estimates were not fully robust, and therefore neutral divergence through genetic drift cannot be ruled out as an alternative explanation. Hatching date showed significant evidence of local adaptation ($P_{ST} > F_{ST}$), although environmental carry-over effects cannot be entirely excluded.

Signal crayfish exhibited very low P_{ST} values and no significant growth variation under common-garden conditions, indicating no genetic divergence in growth and suggesting that observed body size declines reflect environmentally induced plasticity or anthropogenic factors such as harvest pressure rather than local adaptation.

Together, these findings show that local adaptation is a temporally dynamic process, shaped by colonization history and environmental variation. The contrasting patterns between noble crayfish and signal crayfish demonstrate how the relative importance of local adaptation and phenotypic plasticity in life-history traits differs between long-established native populations and recently introduced invasive populations, consistent with theoretical expectations. These findings have implications for management, supporting the use of local populations in noble crayfish restocking and emphasising the need for further research on the drivers of body size declines in signal crayfish populations.

Keywords: Noble crayfish, Signal crayfish, Local adaptation, P_{ST}-F_{ST}, Common-garden experiment, Phenotypic plasticity, Fisheries management

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Abbreviations

Abbreviation	*Description*
SGR	Specific growth rate
SLU	Swedish University of Agricultural Sciences
YOY	Young-of-the-Year

Glossary

Term	*Description*
Adaptive divergence	Evolutionary process, where populations evolve different trait characteristics in response to their environment
Additive genetic variance	Component of genetic variance in a trait that is attributable to the cumulative additive effects of alleles across multiple loci
Carry-over environmental effects	Environmental conditions experienced during an earlier life stage that persist and influence an individual's phenotype at a later stage, independently of the individual's own current environment or genotype
Colonization history	Historical process by which a species has established and spread across a landscape, including the duration and origin of population establishment, which shapes the current geographic distribution, genetic diversity and population structure of present-day populations
Divergent selection	Form of natural selection that favours different phenotypes in different environments, driving populations to diverge in traits over time and representing the primary mechanism underlying local adaptation
Epigenetic effects	Environmentally induced modifications to gene expression that can be transmitted from parents to offspring, arising from changes in how the DNA is expressed rather than changes to the DNA sequence itself
F_{ST}	Index of genetic differentiation between populations or groups based on selectively neutral genetic markers
Genotype	Genetic composition of an individual

Maternal effects	Effect on offspring phenotype that are caused by the maternal environment or maternal phenotype, rather than by the offspring's own genotype
Narrow-sense heritability	Proportion of phenotypic variance in a trait that is attributable to additive genetic variance, representing the fraction of trait variation that is transmitted from parents to offspring through genes
Neutral divergence	Process by which populations diverge genetically through random processes such as genetic drift rather than through natural selection
Pedigree information	Detailed records of the ancestry and family relationships among individuals within a population, allowing the estimation of additive genetic variance and heritability by tracking how traits are transmitted across known generations
Phenotypic plasticity	Ability of a single genotype to produce different phenotypes in response to different environmental conditions, without changes to the underlying DNA sequence
Polygenic	Describing a trait whose phenotypic variation is influenced by multiple genes
P_{ST}	Index of phenotypic trait differentiation between populations, calculated from within and between population phenotypic trait variance
Q_{ST}	Index of quantitative trait divergence between populations, calculated from additive genetic variance within and between populations
Quantitative trait	Trait that varies continuously within a population rather than falling into discrete categories, typically influenced by multiple genes and environmental factors
Stabilizing selection	Form of natural selection favouring similar phenotypes across different populations, maintaining shared trait optima despite genetic drift; the opposite of divergent selection

1 Introduction

Local adaptation is the process by which natural selection increases the relative fitness of local genotypes in their home environment compared to genotypes originating from other environments (Kawecki & Ebert 2004; Hereford 2009). While environmental heterogeneity determines the selective pressures acting upon the populations, genetic variation and population genetic processes determine the capacity to respond to selection and adapt, with high levels of genetic variation facilitating the rate of adaptive change (Hereford & Winn 2008; Fraser et al. 2011).

Life-history traits such as growth rate, age at maturity and fecundity are closely tied to fitness and are therefore common targets of natural selection and local adaptation (Stearns 1998). These quantitative traits are characterized by continuous variation among populations and are polygenic. Selection acts on these traits and the evolutionary response depends on the amount of additive genetic variance. Additive genetic variance reflects the combined additive effects of alleles across loci (Falconer & Mackay 1996). This component of genetic variation determines the extent to which phenotypic variation is heritable and shapes the adaptive potential of a given trait.

Population genetic processes such as genetic drift can reduce adaptive potential by eroding genetic variation or fixing deleterious alleles, thereby lowering fitness and increasing extinction risk (Whitlock 2003). Conversely, gene flow may constrain local adaptation by homogenizing allele frequencies among populations, counteracting divergent selection (García-Ramos & Kirkpatrick 1997).

Several approaches have been developed to study local adaptation in animals and plants. Reciprocal transplant experiments move individuals among native and foreign environments, and local adaptation is inferred when individuals perform best in their home environment (Kawecki & Ebert 2004). While transplant experiments have the advantage of demonstrating overall fitness differences, they do not identify which traits serve these fitness advantages (Savolainen et al. 2013). Furthermore, they may confound genetic adaptation with phenotypic plasticity or maternal effects, and can pose logistic challenges, particularly in animals (Hereford 2009; Savolainen et al. 2013).

Spitze (1993) introduced the Q_{ST}–F_{ST} framework as a method to assess population differentiation in quantitative traits. This framework compares quantitative trait divergence (Q_{ST}) with selectively neutral molecular differentiation (F_{ST}). Q_{ST} is calculated as:

$$Q_{ST} = \frac{\sigma_{AB}^2}{\sigma_{AB}^2 + 2\sigma_{AW}^2}$$

where σ_{AB}^2 represents additive genetic variance among populations and σ_{AW}^2 represents additive genetic variance within populations. When $Q_{ST} > F_{ST}$, divergent

selection is inferred; when $Q_{ST} < F_{ST}$, stabilizing selection is suggested; and when $Q_{ST} \approx F_{ST}$, differentiation may be explained by neutral divergence such as genetic drift.

Robust estimates of Q_{ST} require individuals from different populations to be reared under common environmental conditions to avoid environmental effects on the studied traits. Consequently, Q_{ST}–F_{ST} comparisons are typically combined with common-garden experiments, in which individuals from different populations are reared under identical, controlled environmental conditions, allowing trait differences to be attributed primarily to genetic rather than environmental effects (De Villemereuil et al. 2016).

Since estimating Q_{ST} requires pedigree information to calculate the additive genetic variance within a population, P_{ST}, a phenotypic analogue of Q_{ST}, offers a practical alternative when pedigree data are unavailable. P_{ST} approximates the within-population additive genetic variance of Q_{ST} using the within-population phenotypic variance of a trait, scaled by the narrow-sense heritability. While P_{ST} provides a useful approximation, it should be interpreted with caution as it may conflate additive genetic variance with other sources of phenotypic variance at both within and between population levels, such as maternal or carry-over environmental effects, potentially leading to under- or overestimation of true genetic differentiation (Brommer 2011).

The Q_{ST}-F_{ST} comparison (or P_{ST}-F_{ST} when pedigree information is unavailable) offers several advantages over reciprocal translocation experiments. First, the Q_{ST}-F_{ST} framework is trait-specific, enabling the identification of particular traits that exhibit signs of divergent selection, rather than detecting local adaptation as a general pattern (Leinonen et al. 2013). Second, Q_{ST} relies on common-garden experiments, making it more suitable for studies involving mobile organisms (Kawecki & Ebert 2004). Third, Q_{ST}-F_{ST} analyses can reveal local adaptation even when fitness consequences are subtle or difficult to quantify directly in the field (Whitlock 2008). It should be noted, however, that this approach detects trait divergence rather than local adaptation in the strict sense, as local adaptation requires demonstrating fitness trade-offs across environments. Consequently, a Q_{ST}-F_{ST} analysis and translocation experiments should be viewed as complementary rather than alternatives as reciprocal translocation remains essential for demonstrating fitness advantages of native populations in their environments (Whitlock 2008; Savolainen et al. 2013).

Comparisons between ecologically similar native and invasive species offer a unique perspective to study the temporal dynamics of local adaptation (Ghalambor et al. 2007). Native species have evolved in their habitats for sufficient time to allow for development of local adaptations (Williams & Dawkins 2019). In contrast, invasive species have a shorter colonization history and potentially less time for adaptive divergence, which could limit the extent of local adaptation, although

evolutionary rates can vary between species (Gingerich 1983; Prentis et al. 2008). Additionally, invasive species may possess a limited potential for adaptation due to episodes of reduced population size ("genetic bottlenecks") during the introduction process, which leads to the loss of genetic variation (Dlugosch & Parker 2008). It is generally thought that invasive species usually respond with phenotypic plasticity in the beginning of the colonization event to survive in the new environment (Yeh & Price 2004; Davidson et al. 2011). However, less is known about the role of local adaptation in invasive species, particularly for animals.

Freshwater crayfish in Sweden present a compelling case for comparing a native and an invasive crayfish species occupying similar ecological niches. The native noble crayfish (*Astacus astacus*), formerly widespread and valued as a cultural food resource (Bohman & Edsman 2011), is currently classified as critically endangered (SLU Artdatabanken 2025). While habitat loss and acidification have contributed to the sharp decline, the main threat to the species has been the crayfish plague caused by the oomycete *Aphanomyces astaci*, spread over Europe by introduced North American crayfish species (Martín-Torrijos et al. 2019).

The decline of noble crayfish in Sweden was further accelerated by the introduction of the invasive signal crayfish, *Pacifastacus leniusculus* (Bohman et al. 2006), which acts as a vector for the crayfish plague (Unestam 1969). Contemporary management practices for noble crayfish focus on restoration and restocking (Edsman & Schröder 2009; Bohman & Edsman 2011). Noble crayfish populations are found along substantial latitudinal gradients in Sweden (Albrecht 1983; Souty-Grosset et al. 2006; Bohman 2026), which exposes them to different thermal regimes and growing season lengths. These environmental differences are expected to influence the feeding ecology of noble crayfish, as food availability and feeding activity vary with temperature and season along the latitudinal gradient. Noble crayfish are an omnivorous and opportunistic species, but their dietary composition differs between life-stages. Juvenile noble crayfish feed more on invertebrates, whereas adults show higher level of cannibalism and plant-based food intake (Skurdal & Taugbøl 2002; Veselý et al. 2020), making juveniles particularly vulnerable to seasonal food limitation, which intensifies along latitudinal clines with shorter and more pulsed productive seasons.

Latitudinal gradients have been shown to drive local adaptation in life-history traits of ectotherms, where populations facing different seasonal constraints evolve different strategies of resource allocation to growth, survival and reproduction (Conover & Schultz 1995; Yamahira & Conover 2002; Villeneuve et al. 2021). Local adaptation in growth, where high-latitude populations evolve faster intrinsic growth rates to compensate for shorter growing seasons, is described as countergradient variation and is well documented across fish and amphibian species (Conover & Present 1990; Conover & Schultz 1995; Palo et al. 2003). While countergradient variation is commonly observed in ectotherms, the picture in

crustaceans is less clear. Some evidence supports countergradient variation in growth, as documented in brown shrimp (Campos et al. 2009), while other crustaceans and marine invertebrates show cogradient variation, where genetic effects reinforce rather than compensate the environmental gradient (Lonsdale & Levinton 1985; Trussell 2000). Unlike other ectotherms, growth in crustaceans is discontinuous and represents a complex interaction between moult increment (size increase per moult) and the intermoult period (time between moults) (Hartnoll 2001). Rapid growth therefore requires more frequent moulting, which has been shown to increase vulnerability to cannibalism and mortality in crayfish (Brewis & Bowler 1983; Taugbøl & Skurdal 1992; Kozák et al. 2009; Kouba et al. 2010), potentially affecting how selection acts on growth trajectories along latitudinal clines.

Beyond growth, local adaptation along latitudinal clines has also been documented in breeding phenology across ectotherms. Cogradient variation in breeding phenology is commonly described, where low-latitude populations are genetically predisposed to breed earlier than their conspecifics from higher latitudes (Phillimore et al. 2010; Sunde et al. 2019; Villeneuve et al. 2021).

Swedish noble crayfish populations exhibit strong genetic structuring as inferred from selectively neutral microsatellite markers, with three main population clusters exhibiting different average levels of genetic variability along a north-south gradient, presumably reflecting different postglacial colonization routes (Gross et al. 2013; Dannewitz et al. 2021). The present genetic population structuring may facilitate an adaptive potential. Together with the strong climatic variation, this suggest that selection may have driven divergence in growth rates and breeding phenology among Swedish noble crayfish populations.

Signal crayfish, introduced to Sweden in the 1960s (Svärdson 1965; Fürst 1977), have largely replaced the noble crayfish in southern and central Sweden and are widely harvested in local fisheries. Nowadays, the management of signal crayfish focuses on sustainable harvest, and preventing its further spread (Bohman et al. 2011; Havs- och vattenmyndigheten 2020).

Monitoring programs in Sweden document declining body sizes in certain signal crayfish populations (Rogell et al. 2025). It remains unclear whether this pattern represents environmentally induced phenotypic plasticity (such as density-dependent food limitation), local adaptation to environmental conditions or size-selective harvest pressures. The latter explanation has in many cases been linked to adaptive divergence due to fisheries-induced evolution, in which intense fishing pressure, that targets the largest individuals, selects for faster life histories such as earlier maturation and reduced adult body size (Heino et al. 2015).

While signal crayfish populations in Sweden are expected to face different selective pressures due to fishing and environmental conditions, less is known about their genetic capacity to respond to them. To date, no assessments of the genetic

structure among Swedish populations of signal crayfish using nuclear markers have been made. However, high mitochondrial diversity observed among Swedish populations, likely reflecting the cryptic diversity and multiple genetic lineages present in the species' native range (Larson et al. 2012) as well as several introduction events (Petrusek et al. 2017), may indicate that populations have not been subjected to severe genetic bottlenecks. A comparative approach to assess the genetic structure of Swedish signal crayfish can be made by examining the results of a population genetic structure study conducted by Robinson et al. (2018) in Great Britain. There, weak to strong microsatellite-based genetic differentiation (pairwise F_{ST}: 0.01-0.29) and moderate to high levels of genetic variation (mean expected heterozygosity: 0.5-0.7) were documented among signal crayfish populations. Given that Swedish signal crayfish served as the secondary source for further introductions in Europe (Brinck 1977), including some British populations (Henttonen & Huner 2017), this implicates that Swedish populations may possess similar levels of genetic structuring.

In general, local adaptation in crayfish remains largely unexplored. To date, only one study has addressed this topic using a P_{ST}-F_{ST} framework without common-garden experiments (Lang et al. 2021), which makes it difficult to disentangle observed trait divergence from local adaptation or environmentally induced plasticity. The Swedish system therefore offers a unique opportunity to investigate local adaptation in a pair of native and invasive crayfish species, enabling the assessment of responses to selective pressures across different temporal scales. Additionally, distinguishing between local adaptation and phenotypic plasticity is essential in this context, as the outcomes have direct implications for the development of appropriate restocking strategies for noble crayfish and sustainable harvest management of signal crayfish.

By combining common-garden experiments with P_{ST}-F_{ST} analyses, this master's thesis investigates three hypotheses concerning local adaptation: whether (i) growth and (ii) hatching date are locally adapted in noble crayfish populations, and whether (iii) growth differences among signal crayfish populations are genetically based in a manner consistent with local adaptation.

Together, these analyses enable a comparative assessment of local adaptation in a native and an invasive species, providing insight into how different colonization histories may influence adaptive divergence. For signal crayfish, the experimental design allows an evaluation of whether the observed decline in body size reflects adaptive divergence or phenotypic plasticity in response to local environmental conditions. In addition, survival was examined in both species as differential survival under common-garden conditions may provide supplementary evidence of local adaptation in fitness-related traits.

2 Material and methods

2.1 General Overview

Noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) were studied using the same overarching framework. Young-of-the-year (YOY) individuals originating from multiple populations were reared under common-garden conditions. To assess whether quantitative trait divergence among populations exceeds expectations under neutral genetic differentiation (F_{ST}), divergence in such traits is commonly quantified using Q_{ST} (Leinonen et al. 2013).

For noble crayfish, pedigree information was not available due to unsuccessful parental assignment, which prevented the calculation of the within population additive genetic variance and thus estimation of Q_{ST}. Pedigree information was missing also for signal crayfish, precluding a Q_{ST} estimation for this species as well. Therefore, P_{ST} was employed to quantify population-level trait differentiation. The following formula by Brommer (2011) was used to estimate P_{ST}:

$$P_{ST} = \frac{c * \sigma_B^2}{c * \sigma_B^2 + 2 * h^2 * \sigma_W^2}$$

where σ_B^2 and σ_W^2 represent the phenotypic variance between and within populations, respectively. P_{ST} incorporates the narrow-sense heritability (h^2) to represent the proportion of the phenotypic variance within populations that is due to additive genetic effects. To account for the genetic contribution to phenotypic variance between populations, P_{ST} includes an additional scaling parameter (c), which reflects the proportion of the between population phenotypic variance that is due to additive genetic differences (Brommer 2011). Under common-garden conditions, c is assumed to be 1 (Leinonen et al. 2006; Rogell et al. 2013) as environmental differences are minimised so that phenotypic differences between populations are assumed to primarily reflect genetic differences. Since heritability (h^2) could not be directly estimated, P_{ST} was calculated across a range of h^2 values to evaluate how sensitive P_{ST} estimates are to variation in the genetic contribution to the total phenotypic variation.

$P_{ST} > F_{ST}$ indicates that trait variation among populations exceeds neutral genetic differentiation, suggesting divergent selection. Conversely, $P_{ST} < F_{ST}$ indicates similar selective pressures across populations (stabilizing selection), whereas $P_{ST} \approx F_{ST}$ suggests that observed trait differences could be attributed solely to genetic drift at loci responsible for phenotypic trait variation (Brommer 2011; Leinonen et al. 2013).

For both species, P_{ST} was calculated for growth rate, while P_{ST} for hatching date was only calculated for noble crayfish. Variance components for each respective trait for between and within populations were calculated via a Bayesian linear

mixed model using RStudio (v2025.09.2+418, R Core Team 2025; R package "MCMCglmm", Hadfield 2010). The model generates a posterior distribution of values for the variance components, for both the variance within and between populations, based on a Markov Chain Monte Carlo algorithm (MCMC). Specifications of the MCMC chains differed between models and are described in their respective sections (see 2.2.4 & 2.3.4). Autocorrelation of the posterior samples was low and within the -0.1 to 0.1 interval, and the upper 97.5% quantile of the Gelman-Rubin statistic (R) was below 1.1, indicating that all models successfully converged.

The posterior samples of the variance components were used to calculate P_{ST}. The P_{ST} formula was applied nine times to evaluate the effect of different heritability (h^2) values ranging from 0.1 to 0.9. The resulting P_{ST} values were then represented using the median of the posterior distribution, which is more robust to skewed distributions than the mean. 95% credible intervals were calculated to quantify uncertainty around these estimates.

The comparison of P_{ST} with F_{ST} differed between the species. For noble crayfish, F_{ST} was directly estimated from microsatellite data, whereas the signal crayfish populations examined in this thesis were not genotyped due to financial and time constraints. Consequently, genetic differentiation (F_{ST}) between signal crayfish populations could not be calculated. Instead, F_{ST} values derived from Robinson et al. (2018) were used. Given the shared colonization history of British signal crayfish with Swedish signal crayfish, the British F_{ST} estimates were considered as a reference for possible genetic differentiation scenarios among the studied Swedish signal crayfish populations.

2.2 Noble crayfish (*Astacus astacus*)

The following section presents the data collection and analytical steps used to investigate local adaptation in noble crayfish. The data originated from a study conducted in southwestern Sweden between 2014 and 2018, which was part of a larger originally planned study aiming to establish three rearing environments along the latitudinal gradient of Sweden. By rearing individuals from all populations in each environment simultaneously, this would have functioned as both a common-garden experiment and a reciprocal transplant design, enabling home-site advantage comparisons for the populations included. However, the northern and southern rearing experiments failed, and this thesis uses data from the remaining experimental site located in southwestern Sweden. All statistical analyses and biological interpretations presented here were conducted as part of this thesis.

2.2.1 Study populations and common garden experiment

Five populations from southern to northern Sweden (Figure 1), representing three genetic population clusters (Dannewitz et al. 2021), were selected for the common garden experiment (Table 1). During autumn 2014, before the reproduction season,

females and males from Råneälven, Skellefteälven and Lässerudsälven were transported to a crayfish farm outside the city of Uddevalla in southwestern Sweden (Figure 1) and stocked in three separate 5x5-meter ponds with a depth of approx. 1.5m. In spring 2015, the ponds were drained, and ovigerous females were moved and placed individually in smaller trays (39 × 28 × 14 cm) in the crayfish farm where they were kept until hatching. Due to a lack of ponds for overwintering of crayfish, ovigerous females from the Uddevalla and Torsås populations were collected in spring 2015 and directly moved to individual trays in the crayfish farm. All parental crayfish were measured for morphometric traits, and tissue samples for subsequent genetic population assignment analyses of offspring were taken. In total, 180 ovigerous females were included in the experiment (Table 1).

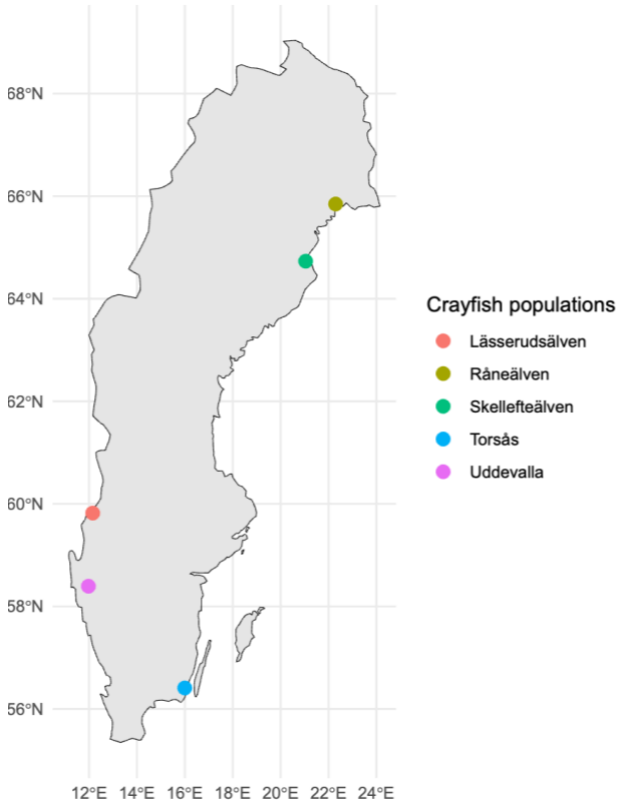

Figure 1 Sampling location of parental noble crayfish used in the common garden experiment (Table 1).

Table 1 Summary of the parental noble crayfish populations used in the common-garden experiment. Population refers to the sampling location. "Cluster" indicates the previously described genetic classification within Sweden (Dannewitz et al. 2021). "N females" represent the number of adult females used for breeding, and "Reproducing" indicates the number of females that produced offspring. "YOY A" and "YOY B" indicate the number of YOY individuals released into each of two ponds used for the common garden experiment in 2015.

Population	Cluster	N females	Reproducing	YOY A	YOY B
Råneälven	Northern	42	15	46	46
Skellefteälven	Northern	25	11	41	49
Lässerudsälven	Central	30	24	100	97
Uddevalla	Southern	40	40	556	555
Torsås	Southern	43	40	194	196
Total		180	130	937	943

From 29 June to 8 July 2015, egg hatching occurred in the trays where ovigerous females were kept, with female-specific hatching date defined as the day when the first freely moving offspring was observed. After hatching, juveniles were maintained for one to two weeks before being stocked into ponds. Early offspring mortality, between hatching and stocking in experimental ponds, was recorded. The initial mortality among juveniles from Råneälven and Skellefteälven was relatively high, probably due to high nitrite levels. Among the 180 ovigerous females, 130 produced offspring that were alive at the onset of the common garden experiment. A small number of hatchlings (10 at maximum) from females with large enough brood size were euthanized and preserved in ethanol. Hatchlings showed no significant differences in size (see Appendix 3).

On 14-15 July 2015, a total of 1890 offspring from 130 parental females representing all five populations were mixed and stocked in two ponds (Table 1), which were equipped with ample perforated brick stones and pieces of electrical conduits for shelter. On 27-28 September 2016, after the second growth season, the ponds were drained to sample noble crayfish individuals. All individuals (n = 196) were sex-determined, and their total body length and weight were measured. Additionally, a small section of a leg was collected for genetic analysis. After sampling, the crayfish were released back into the ponds. The same procedure was repeated in 2018 after the fourth growth season, on 22-23 October 2018. Survival until 2016 and 2018, respectively, was calculated as the proportion of remaining individuals per population relative to the numbers originally released.

2.2.2 Genetic analysis

2.2.2.1 DNA extraction and microsatellite genotyping

Microsatellite genotyping of the parental individuals and the offspring sampled in 2016 had already been performed prior to this project, whereas genotyping of the offspring sampled in 2018 were conducted within this thesis.

DNA was extracted using a chelex protocol (Walsh et al. 1991). Parental crayfish and offspring collected in autumn 2016 were screened for genetic variation at 31 microsatellite loci. Polymerase chain reactions (PCR), following procedures presented in Dannewitz et al. (2021), were carried out using two multiplexes, the first multiplex including 15 loci and the second 16 loci. Primer information including original references is presented in Appendix 1. Electrophoresis was run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with the LIZ 600 sizer. Allele sizes were determined using the ABI Genotyper 3.7 (Applied Biosystems).

Based on results from parental crayfish and offspring from 2016, it was evident that the first multiplex (15 loci) was sufficient to obtain high enough precision in the assignment of offspring to the correct population of origin. Offspring sampled in autumn 2018 were therefore only screened for genetic variation at the 15 loci included in the first multiplex, used in Dannewitz et al. (2021). Further, offspring from 2018 were analysed at a later stage, partly involving new laboratory equipment. Electrophoresis of PCR products from the 2018 offspring was run on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Allele sizes were determined using the software GeneMapper v5.0 (Applied Biosystems). A rerun of a sub-sample of offspring from 2016 using the new equipment revealed a high level of inconsistent results in 3 out of 15 loci due to insufficient genotyping quality, including poor peak resolution, stuttering and ambiguous allele interpretations. The three problematic loci were omitted from further analyses, which means that genetic information from 28 loci was available for subsequent analyses of parental crayfish and offspring from 2016, whereas data from 12 loci were available for offspring sampled in 2018.

2.2.2.2 Genetic differentiation (F_{ST})

No signs of recent stocking and/or admixture in the population samples were found (for more detailed information see Appendix 2). Overall and pairwise genetic differentiation (Weir & Cockerham's F_{ST}) were estimated for the parental generation using the package "hierfstat" (Goudet 2005) in Rstudio (v2025.09.2+418, R Core Team 2025). Parental data were used rather than offspring data, as the origin of the parental generation was known with full certainty. Skellefteälven and Råneälven, both belonging to the northern population cluster, were pooled in the F_{ST} calculations to ensure consistency with the P_{ST} calculation (see 2.2.4), where these two populations had to be pooled due to low survival rates (Table 4). Pairwise comparisons were tested for statistical significance using the R package "genepop" (Rousset 2008).

2.2.2.3 Parental and population assignment

In the parental assignment analysis, it was not possible to assign individuals to their putative parents with a sufficiently high level of statistical confidence (data not shown). Instead, population assignment was performed using the software ONCOR (Kalinowski et al. 2008). Genetic data of offspring sampled in 2016 and 2018 were matched against a baseline consisting of microsatellite genotypes of the parental generation (same individuals as used for F_{ST}-estimation). Population assignment was generally accurate, with assignment probabilities exceeding 90% for most individuals. A small number of individuals showed lower assignment probabilities but were retained for subsequent further analysis (Figure 2). Lower assignment probabilities were primarily associated with individuals sampled in 2018, due to the lower number of genetic markers used in that year (see 2.2.2.1).

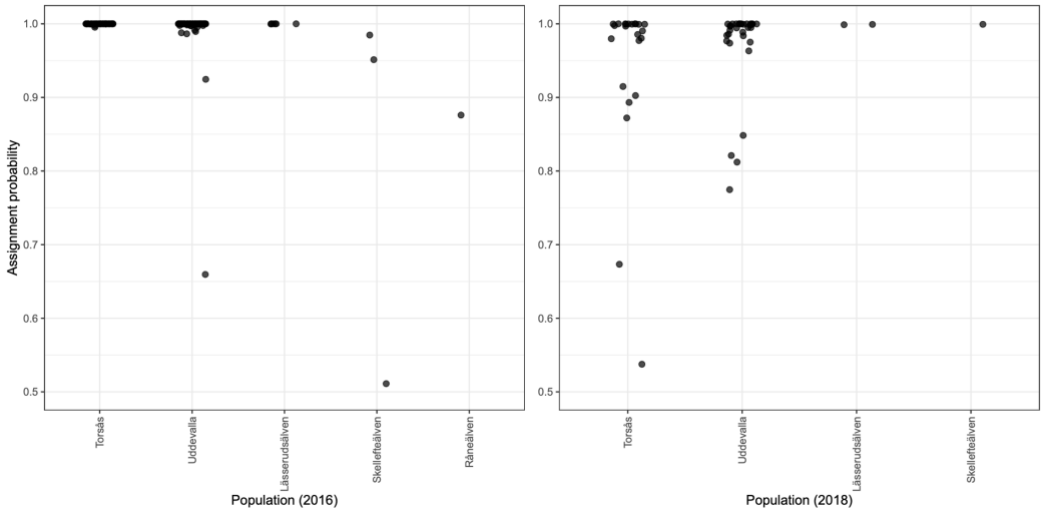

Figure 2 Population assignment probabilities of recaptured individuals for each population in 2016 (left) and 2018 (right).

2.2.3 Trait comparison among populations

Prior to testing for local adaptation, population-level differences were analysed to characterise phenotypic variation among populations. A morphometric analysis of noble crayfish offspring was conducted. This analysis aimed to assess whether phenotypic differences exist between populations.

For offspring, hatching date and total body length for both sampling years (2016 and 2018) were analysed using ANOVA models followed by post hoc comparisons using the R package “emmeans” (Lenth & Piaskowski 2017). The model for body length included sex, population and pond (in which YOY were reared) as fixed effects, as well as the interaction between sex and population. Differences in survival between populations were analysed separately for each time period; survival after the first recapture (2015-16), between the first and second recapture

(2016-18), and the total survival (2015-18) were tested using Fisher's exact test based on the number of surviving and non-surviving individuals.

2.2.4 Quantification of population divergence (P_{ST})

For P_{ST} calculations, total body length of recaptured individuals in autumn 2016 – approximately two growth seasons after initial stocking - was used as a proxy for growth. Size differences in hatchlings preserved in ethanol were examined to validate this approach, confirming that total body length reflects early growth and can serve as a life-history trait (see Appendix 3). Additionally, P_{ST} was calculated for hatching date. Individuals from Skellefteälven and Råneälven (northern genetic cluster) were pooled to increase sample size and improve the robustness of the P_{ST} estimates, because only a small number of individuals from these populations survived until 2016 (Table 4).

In both P_{ST} models for total body length and hatching date, population origin was included as random effect. For total body length, pond was added as fixed effect to account for potential environmental differences between ponds that may have affected growth.

The Markov chain Monte Carlo (MCMC) algorithm consisted of 2,000,000 iterations where the initial 800,000 iterations were removed as burn-in. To minimise autocorrelation, the chain was thinned by retaining every 800^{th} sample. For each trait, three independent chains were run.

2.3 Signal crayfish (*Pacifastacus leniusculus*)

The subsequent sections outline the data collection, experimental design and analytical procedures used for signal crayfish in this thesis.

2.3.1 Study area and sampling methods

For the common-garden experiments, three signal crayfish (*Pacifastacus lenuisculus*) populations (Figure 4) showing stable or decreasing body size trends were selected from different regions in Sweden (Rogell et al. 2025). YOY signal crayfish were captured in the field. Trapping techniques included both passive and active trapping techniques. YOY were collected in September 2025.

Lake Mälaren. Parental crayfish were collected at Lambarudd in Lake Mälaren and maintained at the Institute of Freshwater Research, where YOY included in the common-garden experiment hatched. The habitat at Lambarudd is characterized by a stony substrate at depths of up to four meters and sparse shoreline vegetation. Body size monitoring of this population indicates stable mean body size alongside an increasing size of the largest individuals (Rogell et al. 2025). YOY originated from two different tanks, which had been stocked in May 2025 with six and two ovigerous females, respectively.

Lake Vättern. YOY were caught outside the city of Vadstena. The water depth in the bay ranges between two and five meters. The substrate consists mainly of coarse-grained sand with some rocky patches and was characterized by sparse underwater vegetation. The signal crayfish population in Lake Vättern shows declining average body size (Rogell et al. 2025). Capturing signal crayfish at Lake Vättern, using a small engine-powered boat, was based on two different trap types, which had the same underlying idea of providing burrow habitat for smaller sized crayfish. YOY were caught using either bundles of branches or funnel traps filled with PVC pipes and were subsequently transported to the Research Facility (for more information and catch results see Appendix 4).

Åvaån. A small stream with low discharge located south of Stockholm, characterized by woody vegetation covering the riverbanks and enriching the riverbed with organic material, and habitat structures such as leaves, roots and dead wood. The population in Åvaån has a rather recent colonization history and removal efforts by the fishing rights owners are ongoing (pers. comm. Patrik Bohman, 2026). There is no available information on body size trends. A portable electric fishing device was used to sample a stretch of approximately 50m. Individuals assumed to be YOY (< 35 mm total body length), were retained in a bucket during the sampling period, while larger individuals were released immediately downstream. Transportation to the Research Facility was conducted as described in Appendix 4.

All individuals that were included in the common garden experiment were photographed (fully extended body) on a graph paper, which allowed for multiple

measures on each animal. Morphometric measurements (Figure 3) – of which carapace length measurements were used in subsequent analysis – were conducted with the image analysis software Fiji-4 (Schindelin et al. 2012).

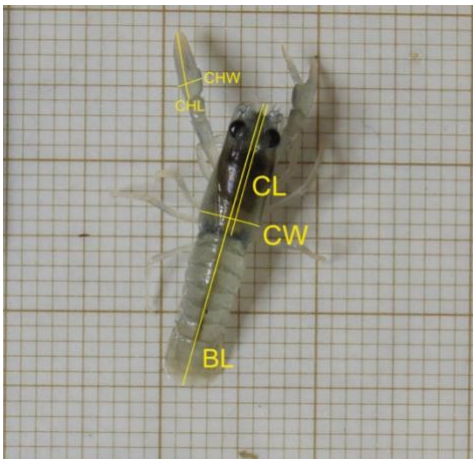

Figure 3 Crayfish with measured traits indicated by yellow lines. BL = Body length; CL = Carapace Length; CW = Carapace width; CHL = Chelae length; CHW = Chelae width.

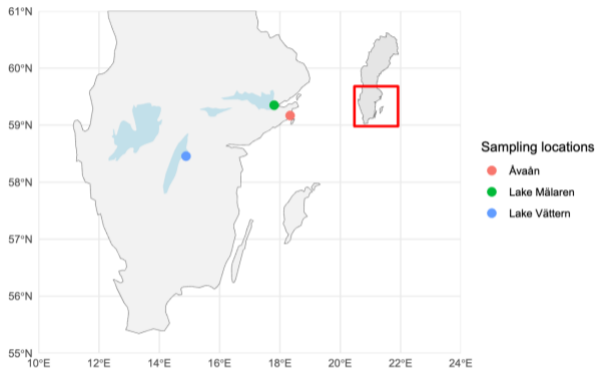

Figure 4 Geographic locations of signal crayfish populations used in the common-garden experiments.

2.3.2 Common garden experiment

In the common-garden experiment, each of 70 13-L aquariums were stocked with one YOY signal crayfish between 12 and 16 September 2025. In total, 13 individuals originating from Lake Vättern, 28 from Lake Mälaren, and 29 from Åvaån were included in the experiment, which was terminated 3 March 2026.

The experiment was conducted with a stratified block design, where the crayfish were divided into five spatial blocks to account for differences in position within the room. Crayfish from the different localities were thereafter distributed in a random manner within blocks, and the temperature was monitored using two temperature loggers per block. For further information on the block design, see Appendix 5. The experiment was conducted in the aquarium facilities at the Institute of Freshwater Research, in a room that is partially affected by the water temperature of Lake Mälaren. This resulted in seasonal shifts in temperature (Table 2).

Table 2 Monthly mean water temperature per block expressed in degrees Celsius. Standard deviation is presented in parenthesis. Note: Measurements for September were excluded due to malfunctioning measuring device.

Block	October	November	December	January	February	March
1	14.01 (0.62)	13.24 (0.93)	11.75 (0.48)	10.01 (0.33)	9.09 (0.52)	9.98 (0.20)
2	14.00 (0.52)	13.14 (0.91)	11.75 (0.46)	10.04 (0.33)	9.13 (0.54)	10.06 (0.28)
3	15.06 (0.27)	14.20 (0.80)	12.75 (0.45)	11.18 (0.25)	10.36 (0.55)	11.36 (0.16)
4	14.99 (0.31)	14.22 (0.84)	12.85 (0.45)	11.22 (0.31)	10.40 (0.59)	11.41 (0.27)
5	14.98 (0.36)	14.23 (0.84)	12.84 (0.52)	11.14 (0.33)	10.47 (0.63)	11.56 (0.34)

The aquariums were equipped with a thin layer of small-grained gravel on its bottom, and one perforated brick fragment as shelter (Figure 5). An oxygen diffusor with a sponge filter was attached to the inner side of each aquarium, to keep water quality high.

Crayfish were fed ad libitum three times per week throughout the experiment using green peas (twice per week) and fish pellets (once per week). Feeding was carried out during regular working hours (8:00-17:00). Each individual received an equal amount of food, consisting of either one frozen pea or ~0.05g of pellets. Water quality was maintained by removing surplus food 1-2 times per week, and performing 20% water changes every second week.

LED panels were used in Block 1-4 to simulate natural light conditions and were programmed to operate on a 12-hour daily cycle. Due to logistic reasons, it was not possible to install the LED panels directly over Block 5, which therefore received light from the nearby blocks, and from two halogen pre-installed lamps programmed to follow the daily solar cycle.

Figure 5 ***(A)*** *Side-view of an individual aquarium equipped with gravel-covered floor, a perforated brick and a sponge filter.* ***(B)*** *Arrangement of Block 5.* ***(C)*** *Side-view perspective of the experimental setup, showing the arrangement of the aquariums. The bottom aquariums represent Block 1, while the aquariums above correspond to Block 3. In the background behind Block 1, Block 2 is visible.*

2.3.3 Trait comparison among populations

Population differences in survival during the experimental period were analysed using Fisher's exact tests based on counts of surviving and non-surviving individuals. As the signal crayfish were collected in nature and hence differed in size at the start of the experiment, total body length could not be applied as a growth measure in contrast to the approach adopted for noble crayfish. Instead, specific growth rate (SGR) was calculated from carapace length. Carapace length was preferred over total body length, because photographing live individuals hampered precise measurement of total body length due to flexing of the abdomen and telson. SGR was analysed using an ANOVA model followed by post hoc comparisons, as described in section 2.2.3, with population and block as fixed effects.

SGR is defined as % growth per day and is calculated as:

$$SGR = (\ln(CL_t) - \ln(CL_i)) * 100/T$$

where CL_t represents the carapace length at the end of the experiment, CL_i = initial carapace length and T = time (days). Only individuals who survived the entire experimental period were included in the calculation.

2.3.4 Quantification of population divergence (P_{ST})

Similar to the noble crayfish, P_{ST} was calculated using the approach described in section 2.1, with SGR as response variable and Population and Block included as random effects. To ensure convergence, the three independent MCMC chain specifications were adjusted for 4,000,000 iterations each, where the initial 800,000 iterations were removed as burn-in. The chains were thinned by retaining every 1800th sample resulting in a posterior distribution of 1778 samples per chain. One individual from Åvaån reared in Block 4 was excluded for the P_{ST} calculation after being identified as an outlier (see Figure 11).

P_{ST} estimates were compared to the range of pairwise F_{ST} comparisons found among British signal crayfish populations by Robinson et al. (2018).

3 Results

3.1 Noble crayfish (*Astacus astacus*)

3.1.1 Genetic analysis

Pairwise F_{ST} values indicate moderate to strong genetic differentiation among populations (Table 3). The overall F_{ST} was 0.33, with a 95% confidence interval ranging from 0.26 to 0.41. Differentiation was lowest between Torsås and Uddevalla (F_{ST} = 0.14) and highest between Torsås and Lässerudsälven (F_{ST} = 0.49). All pairwise comparisons were statistically significant.

Table 3 Results from pairwise comparisons of genetic differentiation, with F_{ST} values above the diagonal and p-values below. Pooled refers to the combined Skellefteälven and Råneälven populations from Northern Sweden. Asterisks (***) indicate statistically significant differences after Bonferroni correction (p<0.001).

Population	Torsås	Uddevalla	Lässerudsälven	Pooled
Torsås	NA	0.14	0.49	0.44
Uddevalla	***	NA	0.31	0.28
Lässerudsälven	***	***	NA	0.29
Pooled	***	***	***	NA

3.1.2 Trait differences among populations

Overall survival of stocked individuals was low, with 3.1 % of initially stocked individuals surviving the full experimental period (Table 4). Survival rate was lower between release and first recapture in 2016 (10.4 %) than from 2016 to 2018 (30%). Survival until 2016 differed significantly among populations (Fisher's exact test, $p < 0.001$), whereas no significant differences were detected for the 2016-18 period (Fisher's exact test, $p > 0.05$). Total survival across the entire period also differed significantly among populations (Fisher's exact test, $p < 0.05$), with higher survival in the southern populations Torsås and Uddevalla compared to the northern populations.

Table 4 Survival of noble crayfish by population between 2015 and 2018. N = number of individuals; S = survival rate. N (2015) = individuals released in 2015; N (2016) and N (2018) = individuals recaptured in 2016 and 2018, respectively; S (15-16) = survival rate between 2015 and 2016; S (16-18) = survival rate between 2016 and 2018; S (15-18) = total survival rate across the full experimental period.

Population	N (2015)	N (2016)	N (2018)	S (15-16)	S (16-18)	S (15-18)
Torsås	390	59	23	15.1%	38.9%	5.9%
Uddevalla	1111	127	33	11.4%	25.9%	3.0%
Lässerudsälven	197	6	2	3.0%	33%	1.0%
Skellefteälven	100	3	1	3.0%	33%	1.0%
Råneälven	92	1	0	1.1%	0%	0.0%
Total	1890	196	59	10.4%	30%	3.1%

Recaptured offspring from Torsås and Uddevalla were generally larger than those from Lässerudsälven, Skellefteälven and Råneälven regardless of year (Figure 6). Overall, males also tended to be larger than females, and this difference was more pronounced in 2018.

In both 2016 and 2018, two-way ANOVA revealed that body length was significantly affected by sex and population, with no significant interaction between sex and population. Pond was only significant in 2016 (Table A6.4 &Table A6.6). Post hoc analyses for 2016 and 2018 indicated population differences among males, particularly in 2016, whereas no significant differences were observed among females. Patterns were consistent across ponds and detailed pairwise comparisons are provided in Table A6.5 andTable A6.7.

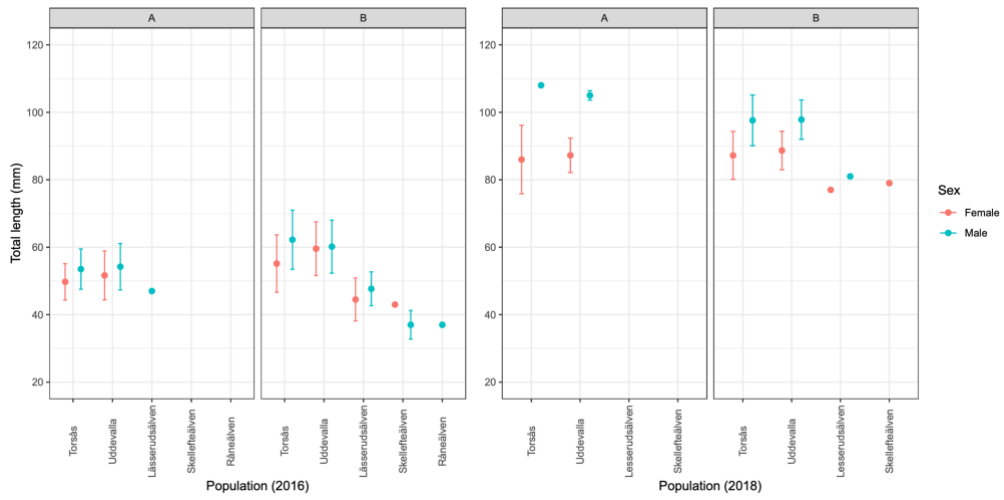

Figure 6 Total body length (mm) of measured individuals across populations and sex in 2016 and 2018 faceted by pond (A and B). Points indicate the mean values for each sex and population group, and error bars represent the corresponding standard deviations. Points without error bars represent single individuals. Full descriptive statistics provided in Table A6.3.

Hatching dates differed among populations (Figure 7). Individuals from the southern genetic cluster (Torsås and Uddevalla) hatched approximately five days earlier than those from the mid and northern clusters (Lässerudsälven, Skellefteälven and Råneälven). A one-way ANOVA test confirmed significant overall differences among populations ($p < 0.05$, Table A6.1). Post hoc comparisons revealed no significant difference in hatching dates between the two southern populations but they both hatched significantly earlier than all mid and northern populations. Lässerudsälven hatching dates differed significantly from Råneälven but not from Skellefteälven. The two northern populations (Skellefteälven and Råneälven) did not differ significantly from each other (Table A6.2).

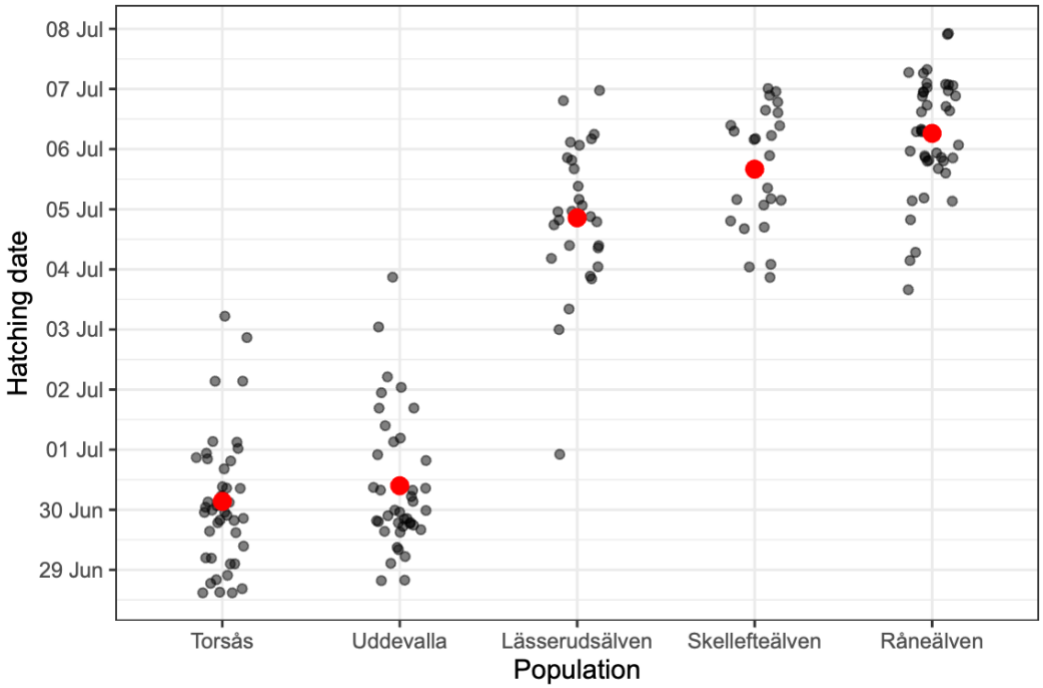

Figure 7 Hatching dates across populations. Grey points show individual observations, and red points indicate population means.

3.1.3 Quantification of population divergence (P_{ST})

P_{ST} estimates for total body length are shown in Figure 8. The P_{ST} estimates varied across the range of assumed heritabilities, with medians ranging from 0.49 to 0.89. Across all heritability values, 95% credible intervals for P_{ST} ranged from 0.15 to 0.61 (lower bounds) and from 0.91 to 0.99 (upper bounds). For low assumed heritabilites (h^2 = 0.1-0.3), P_{ST} estimates were consistently higher than the overall F_{ST}. For higher heritability values, P_{ST} credible intervals overlapped with F_{ST}.

The P_{ST} estimates for hatching date varied between 0.82 and 0.98 across the range of explored heritabilities (Figure 9). The 95% credible intervals ranged from

0.58 to 0.93 (lower bounds), and from 0.97 to 0.99 (upper bounds). For all heritability values, the lower credible intervals were above the overall F_{ST}.

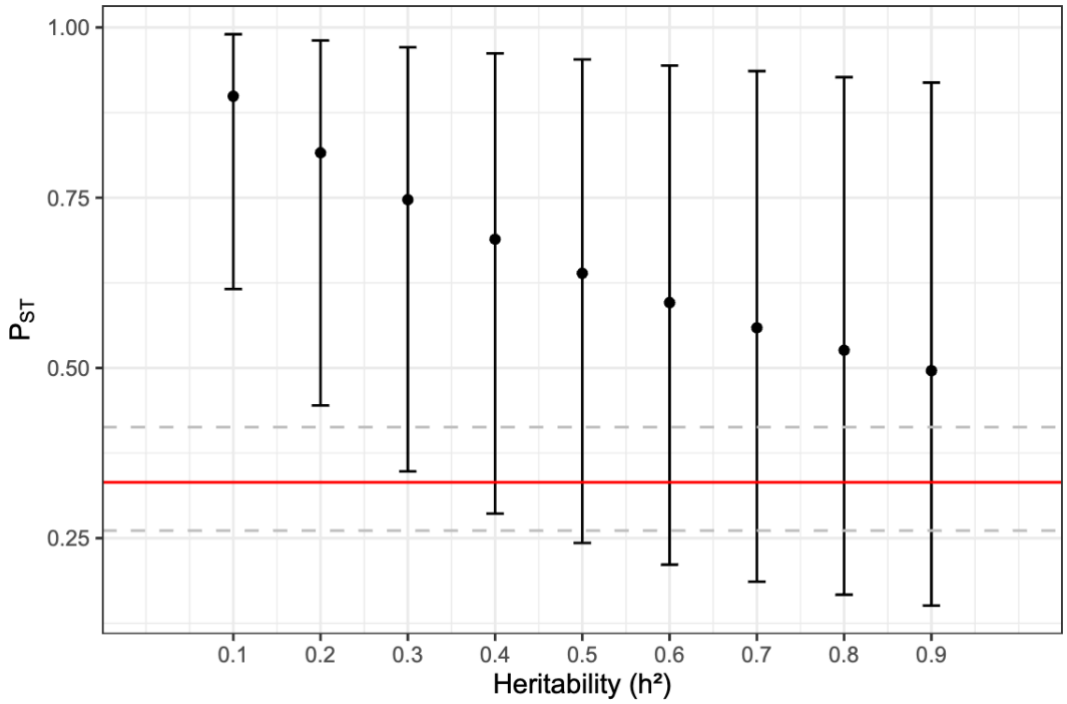

Figure 8 Comparison of P_{ST} estimates for total body length across different assumed values of heritability (h^2). Black points indicate median P_{ST} values for each heritability scenario, with error bars representing the 95% credible intervals. The solid red horizontal line denotes the overall F_{ST}, while dashed lines indicate its 95% confidence interval.

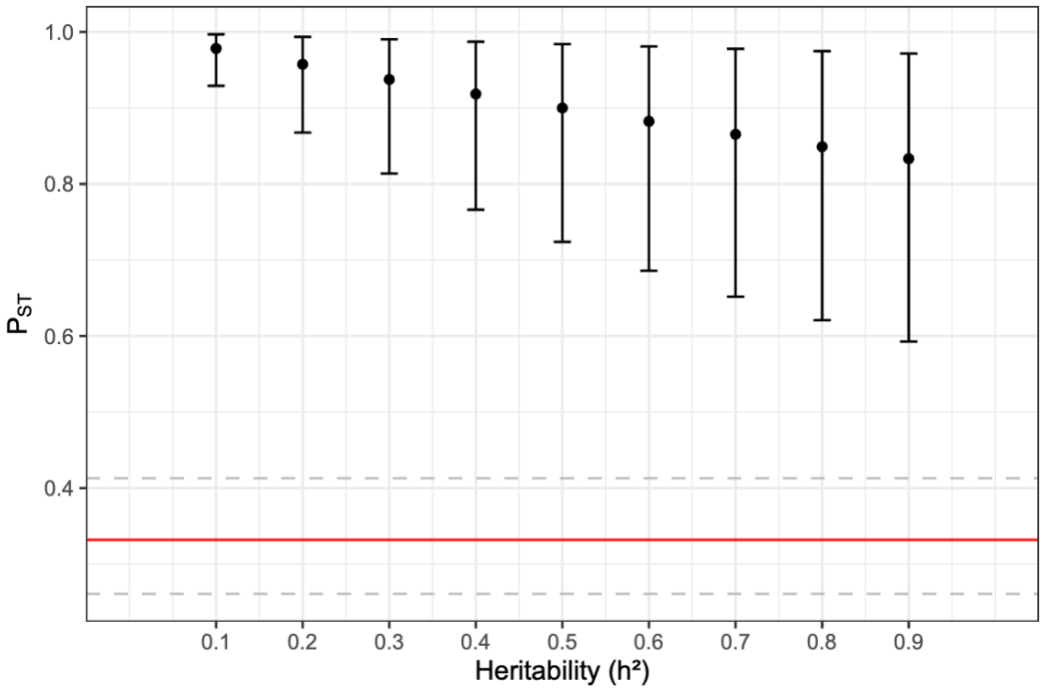

Figure 9 Comparison of P_{ST} estimates for hatching date across different assumed values of heritability (h^2). Black points indicate median P_{ST} values for each heritability scenario, with error bars representing the 95% credible intervals. The solid red horizontal line denotes the overall F_{ST}, while dashed lines indicate its 95% confidence interval

3.2 Signal crayfish (*Pacifastacus leniusculus*)

3.2.1 Trait differences among populations

Survival from initiation to termination of the common-garden experiment was high for individuals from Lake Mälaren and Åvaån, whereas survival of individuals from Lake Vättern was significantly lower (Table 5), as indicated by Fisher´s exact test ($p < 0.05$).

Table 5 Survival of stocked signal crayfish individuals by population.

Population	Stocked	Survived	Survival rate
Åvaån	29	28	96.6%
Lake Mälaren	28	26	92.9%
Lake Vättern	13	9	69.2%

Specific growth rate (SGR) of carapace length did not differ significantly among populations (ANOVA, $p > 0.05$), and no significant block effect was detected ($p > 0.05$, Table A7.2). Mean SGR values were similar across populations, averaging approximately 0.12 % per day (Figure 10). SGR showed substantial variation between individuals within populations (Figure 11).

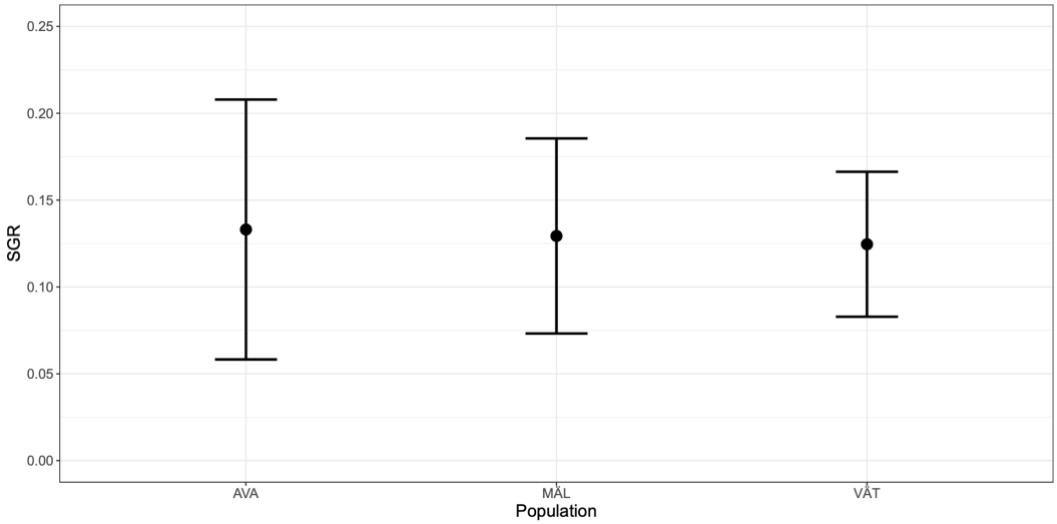

Figure 10 Mean specific growth rate (SGR) in carapace length per population (% per day). Black dots represent population means, and error bars indicate standard deviation. AVA = Åvaån, MÄL = Lake Mälaren, VÄT = Lake Vättern. For exact values see Table A7.1.

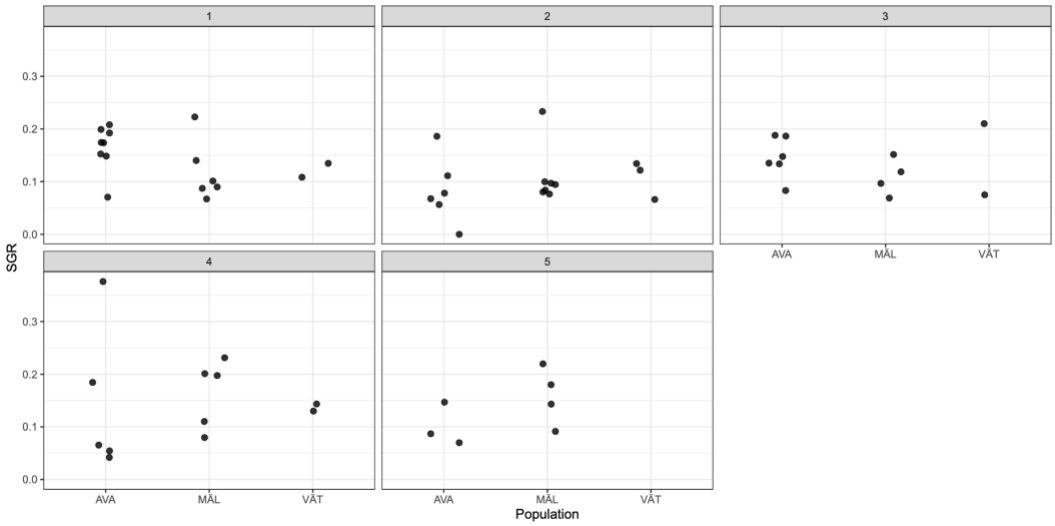

Figure 11 Individual specific growth rates (SGR) in carapace length (% per day) for three signal crayfish population. Data are presented separately for each of five experimental blocks. Points represent individual measurements and are jittered horizontally to improve visibility. For exact values see Table A7.3.

3.2.2 Quantification of population divergence (P_{ST})

The P_{ST} estimates for SGR across the range of assumed heritabilities showed median values close to zero. Upper credible intervals decreased with increasing heritability and remained below 0.23, while lower credible intervals approached zero (Figure 12). For every assumed heritability scenario, the medians were lower than the F_{ST} pairwise comparisons derived from Robinson et al. (2018). Upper credible intervals of P_{ST} exceeded the lower F_{ST} range but were lower than the highest pairwise F_{ST} comparison.

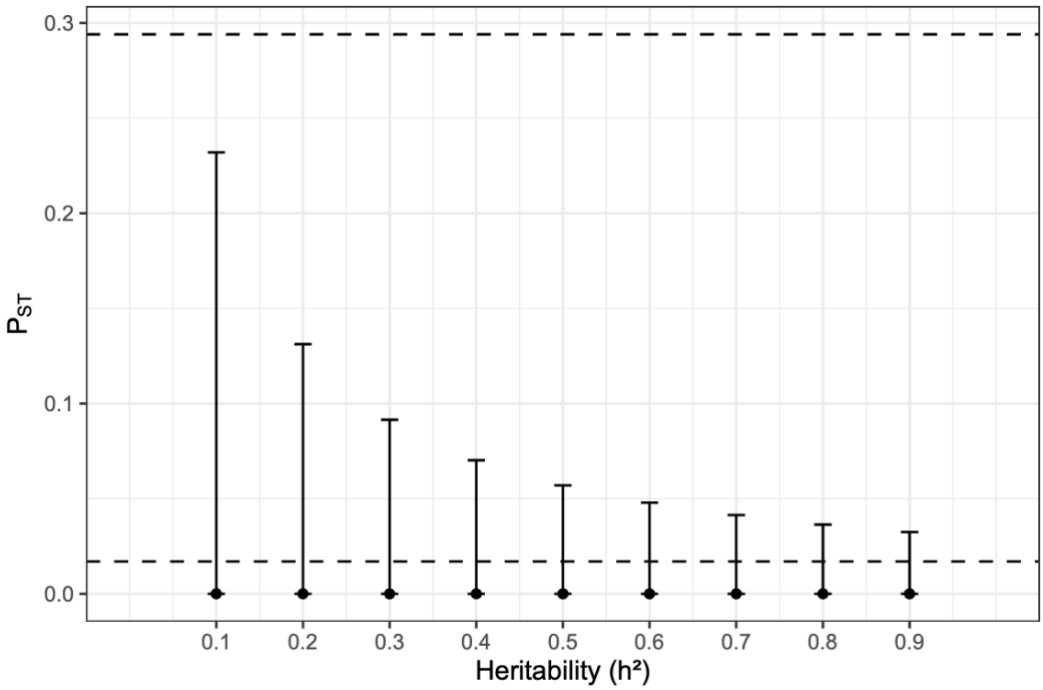

Figure 12 Comparison of P_{ST} estimates for specific growth rate (SGR) in carapace length of signal crayfish across different assumed values of heritability (h^2). Black points indicate median P_{ST} values for each heritability scenario, with error bars representing 95% credible intervals. Dotted lines represent the interval of pairwise F_{ST}-values presented in Robinson et al. (2018).

4 Discussion

This thesis investigated patterns of phenotypic divergence and potential local adaptation in noble and signal crayfish. Using common-garden experiments, growth and hatching date in noble crayfish, and growth in signal crayfish, were analysed in relation to genetic differentiation to disentangle effects of adaptive and neutral processes. Survival was used as supplementary evidence when assessing local adaptation. Due to differences between the species and their respective study designs, the results are discussed separately before being considered from a comparative perspective.

4.1 Noble Crayfish (*Astacus astacus*)

In noble crayfish, total body length (used as proxy for growth) and hatching date were examined across five Swedish populations to assess potential local adaptation along a latitudinal cline. For growth, P_{ST}-F_{ST} comparisons led to different conclusions depending on the assumed heritability values. For low assumed heritabilities (h^2= 0.1-0.3), the lower bounds of the P_{ST} credible intervals exceeded F_{ST}, making divergent selection the most probable inference. This range of heritabilities is particularly relevant, as growth traits in ectotherms are commonly characterised by low heritability (Mousseau & Roff 1987). However, because credible intervals overlapped with F_{ST} across most heritability scenarios, neutral divergence due to genetic drift cannot be excluded.

The observed growth patterns among the studied noble crayfish populations did not align with expectations under countergradient variation as described in other ectotherms (Conover & Present 1990; Palo et al. 2003), since northern and central populations exhibited slower growth than southern populations under common-garden conditions. Instead, the growth divergence among noble crayfish populations is consistent with a cogradient pattern (Lonsdale & Levinton 1985; Trussell 2000; Yamahira & Conover 2002; Villeneuve et al. 2021), which may reflect differences in life-history strategies along the latitudinal cline. Trade-offs between growth and survival have been described across crayfish species, where crayfish species at higher latitudes tend to have slower growth, delayed maturation, and longer lifespans, while species at lower latitudes invest more in rapid growth, early maturation and exhibit shorter lifespans (Momot 1984; Reynolds 2002). The trade-offs between growth and survival are attributed to latitudinal variation in energy availability, where shorter growing seasons and more pulsed energy input at higher latitudes shift energy allocation more towards maintenance and survival to match the more unpredictable environment. In contrast, more stable and productive environments at lower latitudes reduce these energetic constraints, allowing increased allocation towards growth and earlier maturation (Momot 1984).

As the energy availability framework along latitudinal clines has been proposed across crayfish species, the observed divergence in growth between noble crayfish populations may suggest that such variation in life-history strategies can be expressed also within species. Moulting is energetically costly, and more frequent moulting associated with rapid growth increases overall energy expenditure. In northern Swedish environments, selection may instead favour lower basal metabolic rates and reduced energy demand – both consequences of slower growth – allowing individuals to better manage a more pulsed energetic environment and long overwinter periods, during which mortality rates can be substantial due to constrained food availability (Brewis & Bowler 1983). Furthermore, moult mortality risk (unsuccessful moults) increases at low water temperatures (Kouba et al. 2010), meaning that sudden and sharp water temperature drops towards the end of the growing season may make rapid growth trajectories particularly disadvantageous in northern environments.

The generally lower energy availability and more pulsed energetic regimes in northern Sweden may also lead to less productive food webs, potentially weakening selection for rapid growth through reduced predation pressure from gape-size limited predators such as fish, which is consistent with latitudinal predation gradients observed in other ectotherms in Sweden (Laurila et al. 2008). In southern Swedish environments, warmer water temperatures may reduce moulting mortality risk, and higher energy availability sustains higher growth rates throughout a longer growing season (Momot 1984). Here, the fitness benefits of rapid growth may be amplified by higher predation pressure from gape-size limited predators, where outgrowing the vulnerable size window becomes an important fitness advantage (Nyström 2002).

Together, these different environments may have driven the evolution of locally adapted life-history strategies in the noble crayfish populations, where slower growth in the north and faster growth in the south each represent adaptations that maximise fitness under local conditions. Although the observed divergence in growth partially supports this hypothesis, critical life-history traits such as fecundity and age at maturation were not investigated in this thesis, and whether the full suite of life-history trade-offs predicted by this framework is expressed among noble crayfish populations therefore remains an important question for future research. Consequently, the hypothesis (i) that growth in noble crayfish is locally adapted seems probable but was not fully supported by the present results, as evidence was not consistent across all heritability values.

The earlier hatching observed in Torsås and Uddevalla individuals support the prediction of local adaptation in hatching date. The lower credible intervals for P_{ST} estimates were higher than F_{ST} in all heritability scenarios, which suggests that the observed trait divergence is consistent with divergent selection, as it exceeds expectations under neutral differentiation.

The observed divergence in hatching dates likely reflects local adaptation to seasonality and follows a cogradient pattern as identified by other studies on breeding phenology in ectotherms along latitudinal clines (Phillimore et al. 2010; Sunde et al. 2019; Villeneuve et al. 2021; Gotthard et al. 2025). It could be hypothesized that earlier hatching in southern populations reflects phenological adaptations to an earlier onset of productive conditions, matching YOY survival to food availability – particularly the seasonal peak in invertebrate prey, on which juvenile crayfish rely more heavily than adults (Skurdal & Taugbøl 2002; Veselý et al. 2020). In northern populations, later hatching may similarly reflect matching to the later onset of food availability at high latitudes, where hatching before conditions are suitable would expose juveniles to energetically costly environments with limited food (Momot 1984). Overall, the results are consistent with the hypothesis (ii) that hatching date is locally adapted, although further research is required to validate if differences in hatching timing affect fitness across environments, given its potential management relevance for juvenile survival and establishment success in restocking programs.

The divergence in both growth rate and hatching date is consistent with the life-history framework proposed, where northern and southern populations appear to have evolved life-history strategies in response to the contrasting energy environments and seasonality characteristic of their local conditions – with slower growth and later hatching in the north and faster growth and earlier hatching in the south, each representing co-adapted responses that maximise fitness under local conditions. Survival data up to 2016 indicated higher mortality in Lässeruds-, Skellefte-, and Råneälven compared to Uddevalla and Torsås. The higher survival of southern populations in the common-garden experiments, which were located in southwestern Sweden, may provide supplementary evidence of local adaptation analogous to a home-site advantage in a reciprocal transplant experiment.

Together, the results highlight patterns of local adaptation across traits, although both growth and hatching date are influenced by methodological limitations that complicate interpretation. Regarding growth and survival, size-dependent interactions likely influenced growth and survival trajectories, as all individuals were reared together and subject to conspecific interactions within the ponds. The observed growth differences among populations contributed to the survival patterns detected, with faster-growing southern individuals likely gaining a competitive advantage over slower-growing northern individuals. Given the agonistic and cannibalistic behaviour of juvenile noble crayfish, which is often size-dependent with body size influencing the outcome of competitive encounters (Söderbäck 1991), such interactions are likely to occur even under ad libitum feeding conditions and with available shelter (Taugbøl & Skurdal 1992).

Non-lethal competitive interactions may have inflated population differences in growth by reducing resource access among less competitive individuals, while

cannibalism and size-dependent mortality may conversely have led to an underestimation of P_{ST} by selectively removing certain phenotypes and leaving growth trajectories increasingly dominated by competitive survivors. While the magnitude of these contrasting effects cannot be disentangled in the present setup, future research would benefit from rearing crayfish individually, which would provide more accurate estimates of intrinsic growth differences between populations and allow P_{ST} estimates to better reflect the between-population variance. In addition to the uncertainty of the competitive effects occurring in the experimental ponds, the uneven sample sizes among populations could potentially introduce bias in P_{ST} estimates for growth. However, the MCMC approach used is generally robust to such imbalances because uncertainty is expressed through the posterior distribution and is therefore not relying on the assumption of balanced designs (Browne & Draper 2006).

Since the common-garden design was not optimised for studying hatching date, the experimental setup may have introduced biases into the estimates, as environmental or epigenetic effects, reflecting the parental environment rather than the common-garden environment, cannot be excluded. While Lässeruds-, Skellefte-, and Råneälven parental individuals reproduced in ponds at the experimental site in autumn 2014, Uddevalla and Torsås individuals reproduced in their natal environments and were transported to the experimental site in spring 2015. It is possible that higher water temperature in especially Torsås located further south, might have enhanced earlier development of hatchlings from this population, as water temperature is a key determinant of developmental rates in crayfish eggs (Hessen et al. 1987). While this explanation appears plausible for the early hatching date of Torsås crayfish, the Uddevalla females were sampled from a large pond close to the experimental site, and there is reason to assume that water temperature did not differ substantially from those in the ponds where Lässeruds-, Skellefte-, and Råneälven individuals reproduced and overwintered. Thus, the earlier hatching of Uddevalla crayfish partly contradicts the explanation that uncontrolled environmental effects may account for the observed high P_{ST} values.

The results of this thesis have implications for the management and conservation of noble crayfish in Sweden. Evidence of local adaptation in hatching date, and growth, suggests that population origin should be considered in restocking practices. Translocating individuals across large latitudinal gradients risks introducing maladapted genotypes into local stocks, potentially reducing establishment success and long-term population viability. These findings support restocking strategies that prioritise locally sourced individuals to prevent loss of local adaptations and genetic admixture, consistent with the strong genetic structuring observed among Swedish noble crayfish populations in this thesis and in Dannewitz et al. (2021).

However, as local adaptation in this thesis is assessed along a latitudinal gradient, future research could improve restocking strategies, as local adaptation may not always align with latitudinal similarity alone. Local environmental conditions can vary considerably beyond what latitude captures, including variation in thermal regimes and habitat characteristics. Future research examining local adaptation at finer spatial scales, focusing on specific environmental conditions rather than broad geographic gradients, could therefore improve the identification of suitable source populations for restocking programs.

4.2 Signal Crayfish (*Pacifastacus leniusculus*)

The signal crayfish populations studied were analysed to determine whether growth differences may have a genetic basis. The common-garden experiment indicated no significant differences in growth among populations. Given the uncertainty surrounding the F_{ST} estimates, which were based on values reported from local populations in the UK and assumed to represent the studied Swedish signal crayfish populations, the most probable inference is a lack of genetic divergence in growth among populations. P_{ST} medians remained consistently low under all heritability assumptions indicating that divergent selection on growth appears unlikely for any realistic F_{ST} estimate. As a result, the hypothesis (iii), that growth patterns have a genetic basis in a manner consistent with local adaptation, was not supported by the present data.

While the P_{ST} estimates were lower than $F_{ST,}$ this could theoretically be interpreted as stabilising selection ($P_{ST} < F_{ST}$) maintaining similar growth trajectories across populations. However, the differing body size trends observed among the populations – decreasing in Lake Vättern but stable in Lake Mälaren and Åvaån – suggest that growth remains responsive to environmental conditions. Consequently, these differences may more plausibly reflect environmentally induced phenotypic plasticity rather than stabilising selection maintaining a shared genetic growth optimum across populations. This interpretation is consistent with the broad environmental tolerance and high phenotypic plasticity often associated with invasive species (Yeh & Price 2004; Davidson et al. 2011).

Two non-mutually exclusive processes may explain the declining body size in Lake Vättern, i.e. size-selective harvesting and density-dependent limitations. High fishing pressure may have altered the size structure of the populations through the selective removal of large individuals, reducing average body size while simultaneously increasing resource availability for younger year classes (Moorhouse & Macdonald 2011). The resulting increase in abundance may subsequently initiate or intensify density-dependent competition, thereby constraining opportunities for growth and the development of larger individuals (Guan & Wiles 1999).

The relative importance of density-dependent processes versus size-selective exploitation in explaining changes in the abundance and size distribution of signal crayfish remains somewhat unclear. Since larger signal crayfish are typically competitively dominant, and are expected to be less affected by resource limitation than smaller individuals (Harrison et al. 2006), density-dependent competition alone may be insufficient to explain the observed declines, suggesting size-selective harvesting as the more likely primary driver. While these drivers represent the most likely explanations (pers. comm. Björn Rogell 2026), other factors such as altered predation pressure or changes in temperature or water quality may also contribute to plastic responses in growth. Disentangling the relative contributions of these processes requires further research.

Survival was significantly lower in Lake Vättern individuals compared to the other populations. The longer transportation distance for Lake Vättern individuals may have contributed to their reduced survival, though the underlying cause remains unclear. However, unlike the noble crayfish experiment, the controlled conditions of the signal crayfish experiment did not closely resemble the native environment of any population, precluding interpretation of survival differences as evidence of local adaptation in the form of a home-site advantage.

Although the common-garden design aimed to exclude environmental effects, several limitations may have influenced the observed outcomes. Within-population variance in SGR may have been inflated by two sources of variation, which may have contributed to the low P_{ST} estimates observed: carry-over effects from the original environment and temperature-induced differences in moulting stage among individuals at termination.

YOY were collected from wild populations, meaning that maternal and environmental effects could have influenced growth trajectories. While initial size differences have been accounted for using SGR, carry over effects in growth may have persisted throughout the experiment and may partly explain the relatively high within-population variation in SGR. Within-population variation in Åvaån was particularly high due to an outlier in Block 4, and when excluded, variation between Lake Mälaren and Åvaån was relatively similar (data not shown), suggesting that carry-over effects affecting growth were relatively similar. The lower within-population variation in SGR observed in Lake Vättern likely reflects the smaller sample size due to the difficulty of catching YOY rather than being the result of different carry-over effects. Catching juveniles from Lake Vättern was difficult within the available sampling period for this thesis. While both funnel and bundle traps caught YOY (Appendix 4), deployment duration of only a week was insufficient. Generally, both trapping approaches would benefit from extended deployment duration in capturing YOY in future sampling efforts, and bundle traps more specifically, which tended to attract more but larger individuals (Table A4.1),

would benefit from a denser bundle construction to exclude bigger-sized crayfish and in turn enhance YOY catchability.

In addition, suboptimal and variable water temperatures during the experiment may have induced differences in moulting timing among individuals, further inflating within-population variance in SGR. Since moulting is temperature-dependent (Kozák et al. 2009), the relatively low water temperatures in December to February, especially in block 1 and 2 (< 10°C), likely suppressed moulting activity, before the temperature increased and growing conditions became more favourable towards the termination of the experiment. This resulted in individuals being at different stages of their moulting cycle at the time of measurement. SGR may therefore not fully capture intrinsic growth dynamics in this experiment, as it does not account for variation in moulting timing induced by temperature fluctuations. The large number of aquaria and the fact that crayfish consume their old exoskeleton shortly after moulting made systematic monitoring of moulting events logistically unfeasible in the present experimental setup. If temperature-driven differences in moulting dynamics had strongly influenced SGR, this could be expected to result in differences among experimental blocks. No significant block effect on SGR was detected, however, suggesting that such effects did not result in a strong bias in SGR estimates. More broadly, the relatively unfavourable growth conditions during the common-garden experiment may also have reduced the ability to detect, if present, subtle growth differences among populations, thereby limiting the statistical power to identify divergence.

Future growth experiments on crayfish would benefit from monitoring moulting dynamics – such as recording the number of moulting events per individual – alongside the use of climate chambers to maintain water temperatures at levels that promote growth throughout the experimental period, reducing variation in moulting timing. Capturing the entire growth season, rather than terminating the experiment before the end of the growth period, would likely have improved the reliability of the growth estimates. However, this was constrained by the logistical and time limitations of this master's thesis.

The findings of this thesis suggest that environmental rather than genetic factors may be the drivers behind body size declines in Lake Vättern and in other Swedish signal crayfish populations (cf. Rogell et al. 2025). If size-selective harvesting is a contributing factor, this may have broader implications for the management of signal crayfish fisheries across Sweden. However, given the uncertainty surrounding the relative contributions of harvesting pressure and density-dependent processes, further research quantifying harvesting intensity and population dynamics across sites is needed before specific management recommendations could be formulated.

4.3 Comparative Perspective: Native vs. Invasive Crayfish

By comparing local adaptation between noble crayfish and signal crayfish, this thesis provides two snapshots along a colonization timeline, illustrating how adaptive mechanisms differ at different stages. Findings for noble crayfish confirm local adaptation and align with theoretical expectations given their long colonization history. The absence of genetic divergence in growth for signal crayfish is similarly consistent with their shorter colonization history, as approximately 60 years may have been insufficient for genetic divergence to manifest – at least in growth. Given the multi-trait evidence of local adaptation in noble crayfish, this raises the question of whether other traits in signal crayfish might already show early signs of divergence at this stage of colonization.

Whether signal crayfish will follow a similar adaptive trajectory as noble crayfish remains an open question and depends critically on factors that remain uncharacterised. The fine-scale population genetic structure of signal crayfish in Sweden is largely unknown but is expected to play an important role in shaping local adaptation. The degree to which historical bottlenecks have reduced standing genetic variation available for local adaptation remains unclear, while gene flow among populations may have further constrained divergence by homogenising allele frequencies across populations. Furthermore, as an invasive species subject to both environmental gradients and intense harvest pressure, the relative importance of the selective forces acting on signal crayfish remains unclear. If harvest pressure represents a strong selective force, adaptive trajectories in signal crayfish may increasingly be shaped by exploitation-related selection rather than local environmental conditions. Understanding which adaptive trajectory signal crayfish will follow therefore requires both assessment of fine-scale population genetic structure and further investigation of the relative influence of environmental and harvest-related selective pressures.

The comparative native-invasive approach used in this thesis shows that local adaptation is a temporally dynamic process in Swedish crayfish, where plasticity may dominate during early colonization stages before genetic divergence accumulates over longer timescales. The findings also demonstrate the importance of studying multiple traits when assessing local adaptation, as different traits may diverge at different rates and reliance on a single trait alone may provide an incomplete picture of adaptive differentiation.

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Popular science summary

Crayfish not only play an important role in freshwater ecosystems across Sweden but are also of cultural and economic value. However, both native noble crayfish and invasive signal crayfish populations have gone through changes in the past and in recent years. Noble crayfish colonized different climatic regions in Sweden since the end of the last ice age, which can enable population-specific genetic adaptations to local environments. In contrast, some signal crayfish populations consist of smaller individuals over the years, raising questions about whether these changes are caused by the environment or by genetic adaptation.

This thesis studied whether differences between crayfish populations are genetically determined or mainly influenced by the environment. To do this, crayfish from different parts of Sweden were reared under the same controlled conditions, also called common-garden experiments, which allows for separating environmental effects from genetic differences.

For noble crayfish, differences between populations were found in both growth and hatching time. Hatching date was found to be genetically determined, meaning that populations may be adapted to local conditions. Growth seems also to be locally adapted but may be also explained by random genetic processes instead of genetic adaptation.

For signal crayfish, no genetic differences in growth were found under controlled conditions. This suggests that the smaller body sizes observed in the wild are primarily due to environmental factors – such as high population density leading to food shortages and intense fishing – rather than genetic factors.

Overall, the results show that local adaptation in fitness-related traits evolves over time and that, at the beginning of a population's history, these traits can respond more flexibly to local environments. These findings are important for management. For noble crayfish, using local populations in restocking measures may help protect locally adapted traits. For signal crayfish, this thesis shows that further research is needed in order to formulate management implications for declining signal crayfish populations.

Appendix 1 Summary of microsatellite loci used for noble crayfish

Overview of microsatellite loci used in the genetic analysis of noble crayfish, including original references and assigned multiplex groups.

Locus	Reference	Multiplex
Aas4	Koiv *et al*. 2009	1
Aas6	Koiv *et al*. 2009	
Aas9	Koiv *et al*. 2009	
Aas2	Koiv *et al*. 2009	
Aas10	Koiv *et al*. 2009	
Aas3950	Koiv *et al*. 2008	
Aas1198	Koiv *et al*. 2008	
Aas1	Koiv et al. 2009	
Aas5	Koiv *et al*. 2009	
Aas3666	Koiv *et al*. 2008	
Aas7	Koiv *et al*. 2009	
Aas3	Koiv *et al*. 2009	
Aast4_2	Gross *et al*. 2016	2
Aast4_3	Gross *et al*. 2016	
Aast4_7	Gross *et al*. 2016	
Aast4_10	Gross *et al*. 2016	
Aast4_20	Gross *et al*. 2016	
Aast4_17	Gross *et al*. 2016	
Aast4_16	Gross *et al*. 2016	
Aast4_26	Gross *et al*. 2016	
Aast4_30	Gross *et al*. 2016	
Aast4_35	Gross *et al*. 2016	
Aast4_42	Gross *et al*. 2016	
Aast4_32	Gross *et al*. 2016	
Aast4_37	Gross *et al*. 2016	
Aast4_47	Gross *et al*. 2016	
Aast4_46	Gross *et al*. 2016	
Aast4_48	Gross *et al*. 2016	

Appendix 2 Admixture analysis of noble crayfish

Effects of potential recent stocking events, leading to admixture and altered genetic structure of the studied noble crayfish populations, were evaluated by calculating inbreeding coefficients (F_{IS}). Additionally, multivariate analyses (Principal component analysis (PCA); Rstudio package "ade4"), and clustering analyses (Discriminant analysis of principal components (DAPC), R package "adegenet"), were performed to investigate population differentiation and potential admixture.

F_{IS} values across populations were low and varied between -0.001 and 0.044, indicating low levels of inbreeding and that mating within populations is more or less random. The principal component analysis (Figure A2.1), suggests three overall clusters and is therefore consistent with the clustering results reported by Dannewitz et al. (2021). The DAPC analysis showed high overall assignment accuracy (>90%), indicating strong genetic structuring among the predefined groups. Torsås and Uddevalla samples (southern cluster) exhibit slight admixture, with some overlap between samples (Figure A2.2). The pooled northern populations (Skellefte- and Råneälven) form a distinct cluster, as does Lässerudsälven (the central cluster).

Notably, one individual from Lässerudsälven clusters with the pooled populations. Examination of its genotype revealed that this individual was the only one within the Lässerudsälven sample carrying a specific allele at one locus. It remains unclear whether this individual simply carries a rare allele or whether it is due to a genotyping error.

Two individuals from Skellefteälven (displayed as "Pooled" population origin in Figure A2.2) are located in close proximity to Lässerudsälven cluster. DAPC posterior assignment probabilities suggested that these individuals are genetically more similar to Lässerudsälven than to their assigned population. Since genetic sampling took place in spring 2015, it is possible that these individuals moved between overwintering ponds during winter 2014/15 (pers. comm. Stefan Palm, 2026).

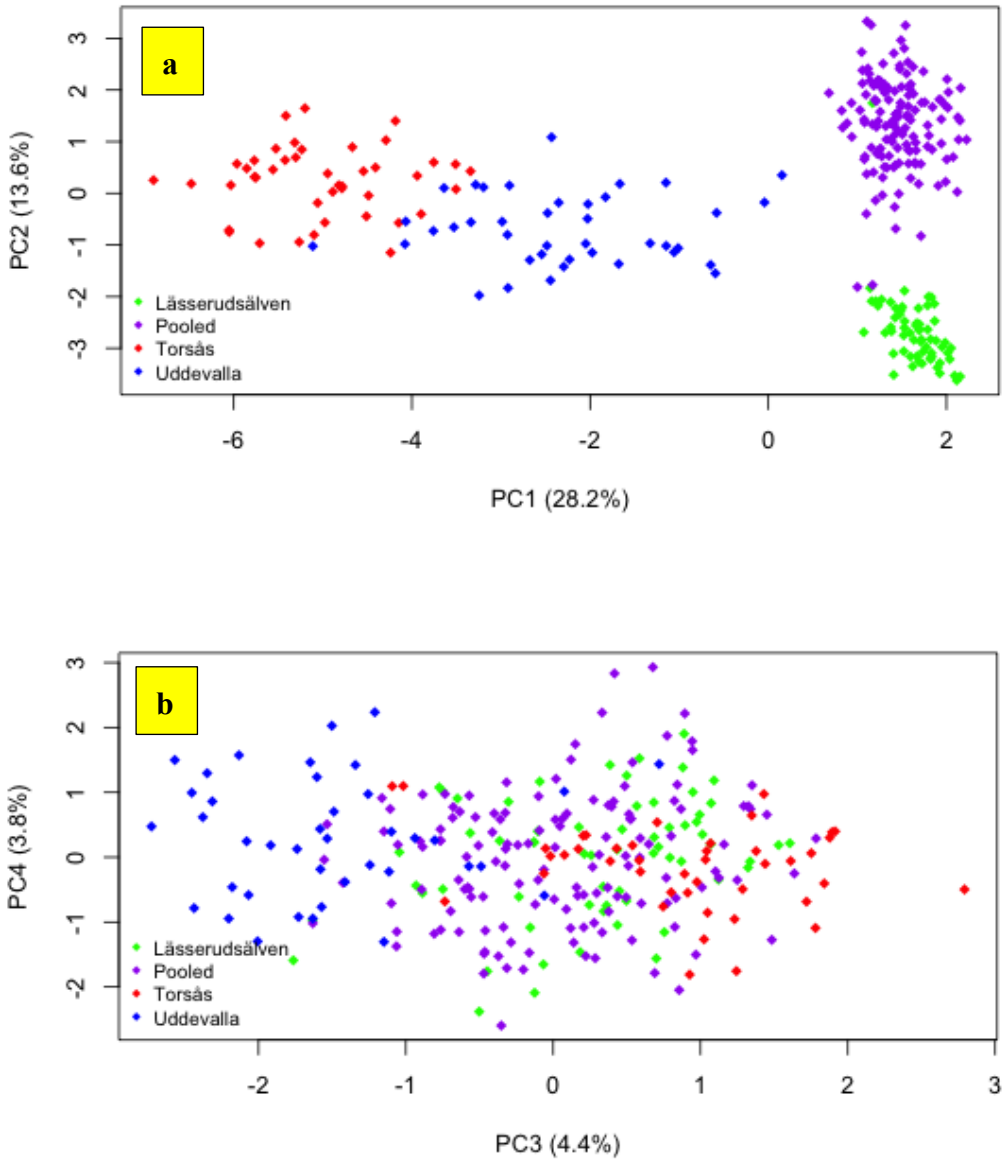

Figure A2.1 Principal Component Analysis (PCA) based on the parental microsatellite genotypes. Figure "a" refers to the principal components 1 & 2; "b" shows principal components 3 & 4. Individuals are coloured by population of origin. Note: "Pooled" refers to the grouped populations Skellefteälven and Råneälven.

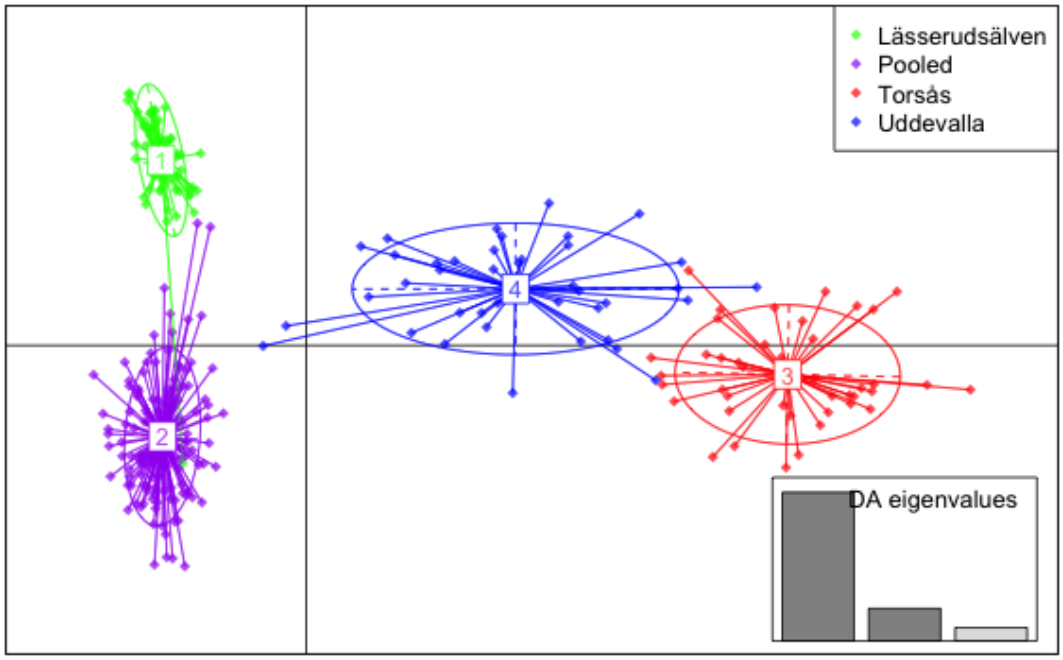

Figure A2.2 Discriminant analysis of principal components (DAPC) based on parental noble crayfish microsatellite genotypes, showing genetic differentiation among predefined populations. Individuals are coloured by population of origin. The first two discriminant functions are shown; ellipses represent within-population genetic dispersion, and the inset scree plot displays the eigenvalues associated with each discriminant axis.

Appendix 3 Analysis of noble crayfish hatchlings

To evaluate if the total body length of recaptured noble crayfish after the second growth season (2016) can be used as a proxy for growth, hatchlings preserved in ethanol were measured as described in Section 2.2.2.2. Measurements revealed that the mean total body length of hatchlings did not differ statistically among populations, justifying the use of total body length as a proxy for growth.

Table A3.1 Mean total body length (mm) of hatchlings among noble crayfish populations. Columns show population, sample size (n), mean total body length (Mean), and standard deviation (SD).

Population	n	Mean	SD
Lässerudsälven	27	10.651	0.499
Råneälven	20	10.560	0.714
Skellefteälven	16	10.730	0.631
Torsås	42	10.728	0.872
Uddevalla	40	10.485	0.804

Table A3.2 Results of one-way ANOVA testing for differences in hatchling total body length among five noble crayfish populations. Shown are the degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F statistic (F value) and significance level (Pr>(F)).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	4	1.486	0.371	0.662	0.619
Residuals	137	76.921	0.561		

Appendix 4 Supplementary information on signal crayfish sampling at in Lake Vättern

To capture signal crayfish YOY, bundle traps constructed from tree twigs were used. The design was inspired by methods described by Nicky Green (www.crayfishuk.org), as well as traditional crayfish-catching techniques practiced by Māori tribes in New Zealand (Kusabs et al. 2018).

Nine bundles (approx. 1m long, 40-50cm diameter) were constructed from deciduous branches (birch, elder, poplar, willow) and tightly bound with 2mm line to prevent entry by larger individuals. To reduce loss of crayfish during retrieval from depths of ~5m, each bundle was fitted with a weighted ground sheet attached to its underside (Figure A.1).

Six bundles were constructed without leaves, while three retained leaves. Leaf presence was expected to increase structural complexity and potentially enhance attractiveness as a dietary cue, given the omnivorous feeding behaviour of crayfish (Guan & Wiles 1998).

Figure A4.1 ***(A)*** *Leaf-containing bundle.* ***(B)*** *Leaf-free bundles.*

In addition, 24 funnel traps were deployed to target small individuals. Each trap contained 40 pipe bundles (seven PVC segments, 70mm length, 16mm diameter) mimicking natural shelters (Figure). Trap entrances were closed to prevent larger crayfish from entering the traps. Smaller crayfish could enter the traps through the mesh sides. Mosquito nets were attached underneath the traps to prevent loss of individuals during retrieval.

Funnel traps were arranged along three buoy-marked lines (two lines with ten traps, one with four traps) at 2-5m spacing and checked on 10 and 12 September 2025. Bundle traps were attached to a single line and deployed for two days. Upon retrieval, traps were processed sequentially to prevent mixing of individuals from different trap types.

Captured crayfish were sorted into two size classes (<35mm and >35mm total length) to reduce cannibalism. Individuals >35mm were released, while smaller crayfish were retained. The 35mm threshold was used as the approximate upper size limit of YOY (P. Bohman, pers. comm., 2025). For each individual total length and trap type were recorded (Table A4.1). Fewer individuals <30 mm, and more individuals >50 mm, were caught in both leafless and leaf-containing bundles. No individuals >60 mm were caught in funnel traps. The higher abundance of larger individuals in bundles likely reflects the bundles' increased attraction, possibly due to dietary cues and better access for larger individuals, while funnel traps, being sealed, prevented capture of individuals >60 mm.

Table A4.1 Catch report from Lake Vättern: Crayfish catch results separated by trap type and size class. Trap abbreviations: LfB = Leaf-free Bundles; LcB = Leaf-containing Bundles; F-Trap = Funnel traps.

Date	Trap type	Size class								Total
		0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	>81	
10.09.25	LfB	0	2	3	0	11	6	9	36	44	111
10.09.25	LcB	0	1	0	0	1	3	2	15	26	48
10.09.25	F-Trap	0	2	4	2	8	1	0	0	0	17
12.09.25	F-Trap	0	1	5	2	11	4	0	0	0	23
Total		0	6	12	4	31	14	11	51	70	199

Retained crayfish were stored in a cooled, moist container with leaves and damp paper towels to maintain humidity and reduce metabolic stress. Standing water was avoided to prevent oxygen depletion during transport (Figure A.3).

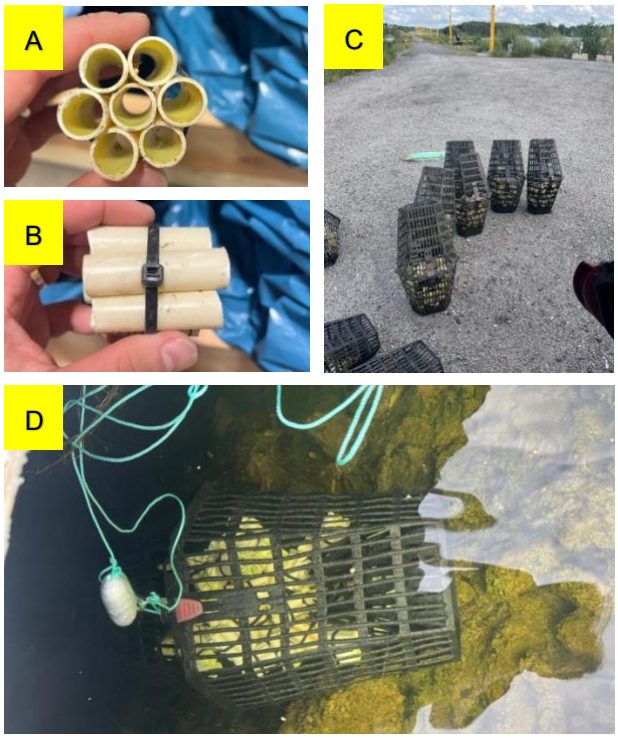

Figure A4.2 ***(A, B)*** *Front and side views of the pipe bundle used in the experiment.* ***(C)*** *Funnel traps equipped with pipe bundles.* ***(D)*** *Funnel trap placed in water.*

Figure A4.3 Polystyrene container used for transportation of crayfish. The cooling pack was wrapped in wet paper towels, and a lid was placed on top to darken the interior of the container.

Appendix 5 Common-garden experiment design for signal crayfish

Presentation of the stratified experimental design for rearing signal crayfish at the Institute of Freshwater Research (Figure A5.1). A stratified design was chosen to ensure an even distribution of populations among experimental blocks, as a fully randomised design would have risked uneven representation of populations within blocks. Aquaria were assigned to block IDs based on their position in the room to account for variation in factors such as light and temperature in the analysis.

Blocks 1-4 were arranged on two-tiered metal shelves (bottom tier = Block 1 and 2; top tier = Block 3 and 4), while Block 5 consisted of aquaria placed in two rearing tanks. Bottom blocks contained 18 aquariums each (36 in total), upper blocks contained 12 aquariums each (24 in total), whereas block 5 contained 10 aquariums, yielding a total of 70 aquariums.

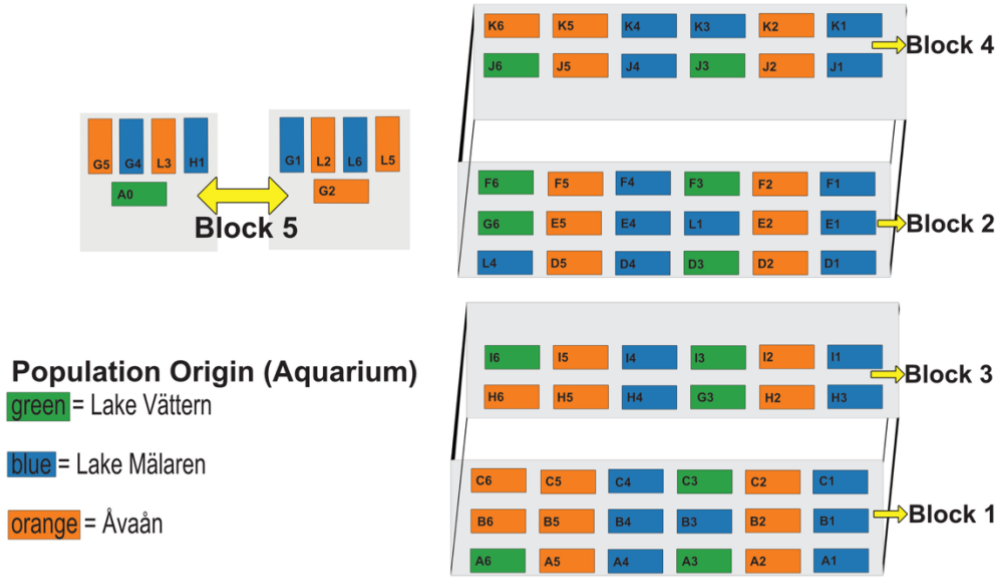

Figure A5.1 Schematic overview of the aquarium arrangement. Colours indicate the population origin of the crayfish in each aquarium, together with their unique tank ID.

Appendix 6 Supplementary information on hatching date and body length of noble crayfish

The tables below present supplementary information from analyses of hatching date and body length (proxy for growth) of noble crayfish, as described in section 3.1.2, from multiple populations reared in common-garden experiments, and measured at two occasions after the second and fourth growth season.

Table A6.1 Results of one-way ANOVA testing for differences of hatching date among five noble crayfish populations. Shown are the degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F statistic (F value) and significance level (Pr>(F)).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	4	1332.5	333.1	284.2	<0.001
Residuals	172	201.6	1.2		

Table A6.2 Results of pairwise Tukey-adjusted comparisons of hatching date among five noble crayfish population. The table shows the estimated difference between population means (Estimate), standard error of the difference (SE), degrees of freedom (Df), t statistic (t.ratio), and Tukey-adjusted p-values (P value).

Comparison	Estimate	SE	Df	t.ratio	P value
Torsås - Uddevalla	-0.26	0.24	172	-1.1	0.809
Torsås - Lässerudsälven	-4.72	0.26	172	-17.94	<0.001
Torsås - Skellefteälven	-5.53	0.28	172	-20.03	<0.001
Torsås - Råneälven	-6.12	0.23	172	-26.06	<0.001
Uddevalla - Lässerudsälven	-4.46	0.27	172	-16.71	<0.001
Uddevalla - Skellefteälven	-5.27	0.28	172	-18.84	<0.001
Uddevalla - Råneälven	-5.86	0.24	172	-24.51	<0.001
Lässerudsälven - Skellefteälven	-0.81	0.3	172	-2.69	0.06
Lässerudsälven - Råneälven	-1.4	0.26	172	-5.32	<0.001
Skellefteälven - Råneälven	-0.6	0.28	172	-2.15	0.205

Table A6.3 Summary of mean total length (mm) and standard deviation (SD) for recaptured noble crayfish grouped by population, sex and pond in 2016 and 2018.

Best Estimate	Sex	Year	Pond	mean_length	sd_length
Lesserudsälven	Female	2016	B	44.5	6.364
Lesserudsälven	Male	2016	A	47.0	NA
Lesserudsälven	Male	2016	B	47.6	5.033
Råneälven	Male	2016	B	37.0	NA
Skellefteälven	Female	2016	B	43.0	NA
Skellefteälven	Male	2016	B	37.0	4.243

Torsås	Female	2016	A	49.8	5.392
Torsås	Female	2016	B	55.2	8.493
Torsås	Male	2016	A	53.5	5.972
Torsås	Male	2016	B	62.2	8.772
Uddevalla	Female	2016	A	51.6	7.239
Uddevalla	Female	2016	B	59.6	7.962
Uddevalla	Male	2016	A	54.2	6.888
Uddevalla	Male	2016	B	60.2	7.850
Lesserudsälven	Female	2018	B	77.0	NA
Lesserudsälven	Male	2018	B	81.0	NA
Skellefteälven	Female	2018	B	79.0	NA
Torsås	Female	2018	A	86.0	10.140
Torsås	Female	2018	B	87.2	7.085
Torsås	Male	2018	A	108.0	NA
Torsås	Male	2018	B	97.6	7.516
Uddevalla	Female	2018	A	87.3	5.131
Uddevalla	Female	2018	B	88.7	5.701
Uddevalla	Male	2018	A	105.0	1.414
Uddevalla	Male	2018	B	97.8	5.845

Table A6.4 Results of a two-way ANOVA testing the effects of sex, population, pond and the interaction sex-population on body length of recaptured noble crayfish in 2016. The table shows degrees of freedom (Df), sums of squares (Sum Sq), mean squares (Mean Sq), F-values, and associated p-values for each model term.

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Sex	1	488	487.9	8.109	0.005
Population	4	2395	598.7	9.950	<0.001
Pond	1	1611	1611.2	26.779	<0.001
Sex:Population	3	336	112.1	1.863	0.137
Residuals	186	11191	60.2		

Table A6.5 Results of pairwise Tukey-adjusted comparisons of offspring body length among five noble crayfish population in 2016, separated by sex and pond. The table shows the estimated difference between population means (Estimate), standard error of the difference (SE), degrees of freedom (Df), t statistic (t.ratio), and Tukey-adjusted p-values (P value).

Comparison	Sex	Pond	Estimate	SE	Df	t.ratio	P value
Lässerudsälven - Råneälven	Female	A	NA	NA	NA	NA	NA
LässerudsIven - Skellefteälven	Female	A	1.5	9.5	186	0.16	0.999
Lässerudsälven - Torsås	Female	A	-11.1	5.69	186	-1.95	0.211
Lässerudsälven - Uddevalla	Female	A	-14.72	5.58	186	-2.64	0.045
Råneläven - Skellefteälven	Female	A	NA	NA	NA	NA	NA
Råneläven - Torsås	Female	A	NA	NA	NA	NA	NA
Råneälven - Uddevalla	Female	A	NA	NA	NA	NA	NA
Skellefteälven - Torsås	Female	A	-12.6	7.9	186	-1.59	0.385
Skellefteälven - Uddevalla	Female	A	-16.22	7.83	186	-2.07	0.166

Torsås - Uddevalla	Female	A	-3.62	1.74	186	-2.08	0.162
Lässerudsälven - Råneälven	Male	A	12.24	8.68	186	1.41	0.622
Lässerudsälven - Skellefteälven	Male	A	12.24	6.73	186	1.82	0.365
Lässerudsälven - Torsås	Male	A	-12.75	4.12	186	-3.09	0.019
Lässerudsälven - Uddevalla	Male	A	-11.09	4.01	186	-2.76	0.049
Råneälven - Skellefteälven	Male	A	0	9.5	186	0	1
Råneälven - Torsås	Male	A	-25	7.88	186	-3.17	0.015
Råneälven - Uddevalla	Male	A	-23.33	7.83	186	-2.98	0.027
Skellefteälven - Torsås	Male	A	-25	5.66	186	-4.41	<0.001
Skellefteälven - Uddevalla	Male	A	-23.33	5.58	186	-4.18	<0.001
Torsås - Uddevalla	Male	A	1.67	1.73	186	0.96	0.871

Lässerudsälven - Råneälven	Female	B	NA	NA	NA	NA	NA
Lässerudsälven - Skellefteälven	Female	B	1.5	9.5	186	0.16	0.999
Lässerudsälven - Torsås	Female	B	-11.1	5.69	186	-1.95	0.211
Lässerudsälven - Uddevalla	Female	B	-14.72	5.58	186	-2.64	0.045
Råneälven - Skellefteälven	Female	B	NA	NA	NA	NA	NA
Råneälven - Torsås	Female	B	NA	NA	NA	NA	NA
Råneälven - Uddevalla	Female	B	NA	NA	NA	NA	NA
Skellefteälven - Torsås	Female	B	-12.6	7.9	186	-1.59	0.385
Skellefteälven - Uddevalla	Female	B	-16.22	7.83	186	-2.07	0.166
Torsås - Uddevalla	Female	B	-3.62	1.74	186	-2.08	0.162
Lässerudsälven - Råneälven	Male	B	12.24	8.68	186	1.41	0.622

Lässerudsälven - Skellefteälven	Male	B	12.24	6.73	186	1.82	0.365
Lässerudsälven - Torsås	Male	B	-12.75	4.12	186	-3.09	0.019
Lässerudsälven - Uddevalla	Male	B	-11.09	4.01	186	-2.76	0.049
Råneälven - Skellefteälven	Male	B	0	9.5	186	0	1
Råneälven - Torsås	Male	B	-25	7.88	186	-3.17	0.015
Råneälven - Uddevalla	Male	B	-23.33	7.83	186	-2.98	0.027
Skellefteälven - Torsås	Male	B	-25	5.66	186	-4.41	<0.001
Skellefteälven - Uddevalla	Male	B	-23.33	5.58	186	-4.18	<0.001
Torsås - Uddevalla	Male	B	1.67	1.73	186	0.96	0.871

Table A6.6 Results of a two-way ANOVA testing the effects of sex, population, pond and the interaction sex:population on body length of recaptured noble crayfish in 2018. The table shows degrees of freedom (Df), sums of squares (Sum Sq), mean squares (Mean Sq), F-values, and associated p-values for each model term.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	1	1628.9	1628.9	39.079	< 0.001
Population	3	462.7	154.2	3.701	0.017
Pond	1	6.2	6.2	0.148	0.702
Sex:Population	2	31.1	15.5	0.373	0.691
Residuals	52	2134.079	41.039		

Table A6.7 Results of pairwise Tukey-adjusted comparisons of offspring body length among five noble crayfish population in 2018, separated by sex and pond. The table shows the estimated difference between population means (Estimate), standard error of the difference (SE), degrees of freedom (Df), t statistic (t.ratio), and Tukey-adjusted p-values (P value).

Comparison	Sex	Pond	Estimate	SE	Df	t.ratio	P value
Lässerudsälven - Skellefteälven	Female	A	-2	9.13	51	-0.22	0.996
Lässerudsälven - Torsås	Female	A	-9.69	6.74	51	-1.44	0.482
Lässerudsälven - Uddevalla	Female	A	-10.19	6.71	51	-1.52	0.434
Skellefteälven - Torsås	Female	A	-7.69	6.74	51	-1.14	0.666
Skellefteälven - Uddevalla	Female	A	-8.19	6.71	51	-1.22	0.617
Torsås - Uddevalla	Female	A	-0.5	2.4	51	-0.21	0.997
Lässerudsälven - Skellefteälven	Male	A	NA	NA	NA	NA	NA
Lässerudsälven - Torsås	Male	A	-17.46	6.75	51	-2.59	0.033
Lässerudsälven - Uddevalla	Male	A	-18.4	6.87	51	-2.68	0.026
Skellefteälven - Torsås	Male	A	NA	NA	NA	NA	NA
Skellefteälven - Uddevalla	Male	A	NA	NA	NA	NA	NA
Torsås - Uddevalla	Male	A	-0.94	3.02	51	-0.31	0.948

Lässerudsälven - Skellefteälven	Female	B	-2	9.13	51	-0.22	0.996
Lässerudsälven - Torsås	Female	B	-9.69	6.74	51	-1.44	0.482
Lässerudsälven - Uddevalla	Female	B	-10.19	6.71	51	-1.52	0.434
Skellefteälven - Torsås	Female	B	-7.69	6.74	51	-1.14	0.666
Skellefteälven - Uddevalla	Female	B	-8.19	6.71	51	-1.22	0.617
Torsås - Uddevalla	Female	B	-0.5	2.4	51	-0.21	0.997
Lässerudsälven - Skellefteälven	Male	B	NA	NA	NA	NA	NA
Lässerudsälven - Torsås	Male	B	-17.46	6.75	51	-2.59	0.033
Lässerudsälven - Uddevalla	Male	B	-18.4	6.87	51	-2.68	0.026
Skellefteälven - Torsås	Male	B	NA	NA	NA	NA	NA
Skellefteälven - Uddevalla	Male	B	NA	NA	NA	NA	NA
Torsås - Uddevalla	Male	B	-0.94	3.02	51	-0.31	0.948

Appendix 7 Supplementary information on specific growth rates in signal crayfish

Additional information on the average specific growth rate in carapace length for signal crayfish, calculated for the populations and experimental blocks in the common-garden experiment referred to in Section 3.2.1, is shown below, including significance tests.

Table A7.1 Mean specific growth rate (Mean SGR) in carapace length (% per day) for each signal crayfish population with standard deviation (SD). N represents the number of individuals in each population.

Population	N	Mean SGR	SD
Åvaån	28	0.133	0.075
Mälaren	26	0.123	0.056
Vättern	9	0.124	0.042

Table A7.2 Results of one-way ANOVA testing for differences in SGR of carapace length among three signal crayfish populations and experimental blocks. Shown are the degrees of freedom (Df), sum of squares (Sum Sq), mean squares (Mean Sq), F statistic (F value) and significance level (Pr>(F)).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	2	0.001	0.002	0.065	0.938
Block	1	0.002	0.002	0.423	0.518
Residuals	60	0.244	0.004		

Table A7.3 Mean specific growth rate (Mean SGR) in carapace length (% per day) for each block with standard deviation (SD). N represents the number of individuals reared in each block.

Block	N	Mean SGR	SD
1	16	0.142	0.051
2	16	0.099	0.053
3	12	0.133	0.046
4	12	0.151	0.095
5	7	0.135	0.054

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