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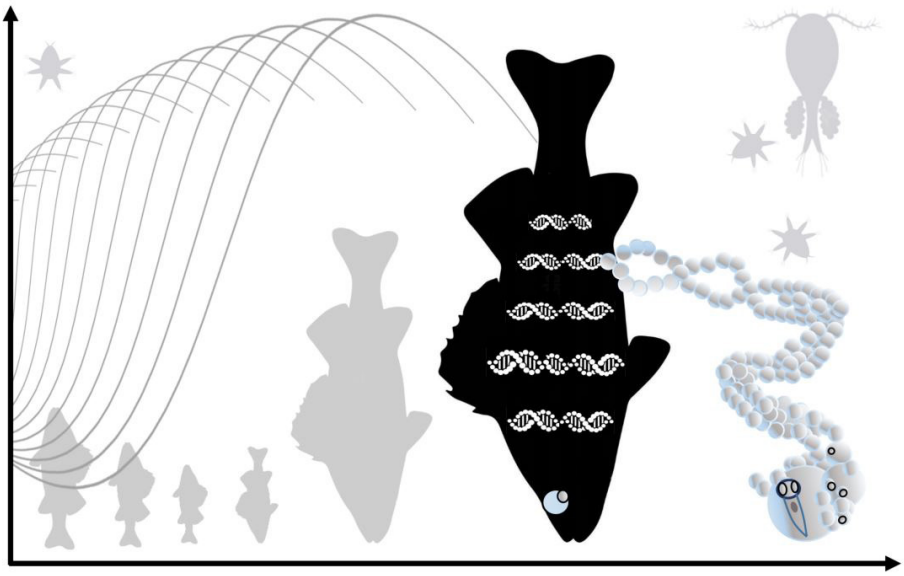
Ecological and evolutionary consequences of ecosystem warming in fish

Jingyao Niu



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Cover: the circle of life for a hypothetical Eurasian perch, from the double helix structure of DNA, the foundation that codes for its traits and functions, to egg, larvae, growth at different sizes and its food.

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Abstract

Climate warming forces ectothermic organisms to shift their spatial or temporal distribution, acclimatize or adapt to new thermal conditions to survive. Fish, as key ectotherms with significant ecological roles and socio-economic values, have been widely studied in this context. However, whether warming induces evolution in wild fish populations over time, and if so, how such evolutionary effects can impact the community and ecosystem more broadly remain largely unknown.

In this thesis, I investigated the effects of ecosystem-warming on fish by comparing traits and genomes of Eurasian perch from an artificially heated area with those from a neighbouring unheated area over four decades. Long-term trait data analyses showed that perch mature earlier and at smaller body sizes with increased reproductive investment in the first generations exposed to warming. After four to eight generations of warming, size at maturation varied more, indicating an evolutionary component in maturation-related trait responses to warming. Utilising a customised DNA extraction protocol, I obtained high-quality whole genome sequencing data for perch from historical archival bones as well as contemporary muscle samples. Selection signatures in single nucleotide variants were revealed between perch from the heated and unheated area over time, suggesting natural selection due to warming. Lastly, I conducted a mesocosm experiment, exposing a pelagic food web module to a temperature gradient using perch larvae from both the heated and unheated areas. Fish from the heated area may have adapted to warming in feeding-related traits, as indicated by differences in their zooplankton prey community composition compared to fish from the unheated area.

Overall, the thesis demonstrates that warming can impose evolutionary and ecological consequences on fish in the wild, and underscores how such consequences can propagate through species interactions and influence other components in the ecosystem. This thesis emphasises the importance of synthesising research on ecology and evolution to better understand how ectotherms respond to rising temperatures and the biological processes involved, especially in the face of intensifying climate warming challenges.

Keywords: climate change, life history trait, maturation, food webs, trophic interactions, ecological genomics, thermal biology, evolutionary biology, Eurasian perch

Ekologiska och evolutionära konsekvenser av ekosystem-uppvärmning hos fisk

Abstract

Klimatuppvärmning tvingar ektotermiska organismer att förändra sin utbredning eller migrationsmönster, acklimatisera eller anpassa sig till nya temperaturer för att överleva. Fisk spelar avgörande roller i ekosystemet och har stort socioekonomiskt värde och många studier har därför gjorts på hur klimatuppvärmning påverkar fisk. Emellertid kvarstår frågan om uppvärmning inducerar evolution i vilda fiskpopulationer över tid, och hur sådana evolutionära effekter kan påverka ekologiska samhällen och ekosystem mer generellt. I denna avhandling har jag studerat fyra decennier av ekosystemuppvärmning i ett artificiellt uppvärmt område. Jag analyserade uppvärmningseffekterna på fisk genom att jämföra egenskaper och genom hos abborre från detta område och ett angränsande ouppvärmt område. Mina analyser av data på egenskaper visade att abborrarna blir könsmogna tidigare och vid mindre kroppsstorlekar samt har ökad reproduktiv investering i de första generationerna som utsattes för uppvärmning. Med tiden varierade storleken vid könsmognad mer, vilket tyder på evolutionära komponenter i dessa egenskapers respons. Genom att använda ett DNA-extraktionsprotokoll som jag utvecklade, erhöll jag högkvalitativa helgenomsekvenseringsdata från arkiverade benprover och samtida muskelprover. Genetiska selektionssignaturer i enbaspolymorfier identifierades hos abborre från det uppvärmda området som skiljer sig från de i det ouppvärmda området vid de tre tidpunkterna, vilket tyder på naturligt urval på grund av uppvärmning. Slutligen genomförde jag ett mesokosmexperiment där jag utsatte en mindre pelagisk näringsväv för en temperaturgradient. Fiskyngel som härstammade från det uppvärmda området kan ha anpassat sin födosökning till följd av uppvärmningen, vilket återspeglades av responsen hos art- och storlekssammansättningen i djurplanktonsamhället som utgör deras byten. Sammanfattningsvis visar avhandlingen att uppvärmning kan leda till evolutionära och ekologiska konsekvenser för fisk i det vilda, och den understryker hur sådana konsekvenser kan spridas genom artinteraktioner och påverka andra komponenter i ekosystemet. Denna avhandling belyser behovet av att syntetisera forskning inom ekologi och evolution för att förstå hur ektoterma organismer svarar på temperaturökning samt de biologiska processer som är involverade i denna tid av intensifierande klimatuppvärmningsutmaningar.

Nyckelord: klimatförändringar, livshistorieegenskaper, könsmognad, näringsvävar, trofiska interaktioner, ekologisk genomik, termisk biologi, evolutionär biologi, abborre

Dedication

To Mom and Dad, Jianping Yang and Xuedong Niu

致我的父亲母亲，牛学东和杨建萍

Be water, my friend (Bruce Lee)

Contents

List of publications

This thesis is based on the work contained in the following papers and manuscripts, referred to by Roman numerals in the text, * signals the corresponding author:

I. Jingyao Niu*, Magnus Huss, Anti Vasemägi and Anna Gårdmark. (2023). Decades of warming alters maturation and reproductive investment in fish. Ecosphere 14:e4381. https://doi.org/10.1002/ecs2.4381

II. Jingyao Niu*, Magnus Huss, Aurélie Garnier, Anti Vasemägi and Anna Gårdmark. (2024). Multi-decadal warming alters predator's effect on prey community composition. Proceedings of the Royal Society B 291:20240511. https://doi.org/10.1098/rspb.2024.0511

III. Jingyao Niu*, Anti Vasemägi, María-Eugenia López, Lilian Pukk, Magnus Huss and Anna Gårdmark. DNA isolation from boiled archival fish bones yields high quality whole genome sequence data. (submitted)

IV. Jingyao Niu*, María-Eugenia López, Anti Vasemägi, Magnus Huss and Anna Gårdmark. Whole genome analyses of archived specimens suggest selection from ecosystem warming in a wild fish population. (manuscript)

The contribution of Jingyao Niu to the papers included in this thesis was as follows:

I. Refined the study design, led data curation, performed formal analyses and visualization, wrote the original draft, and led the revision of the manuscript with contributions from co-authors.

II. Contributed to the conceptualization and experimental design, carried out the experiment and data sampling with the assistance of co-authors, curated the data, performed formal analyses and visualization, wrote the original draft, and led the revision of the manuscript with input from co-authors.

III. Led the methodology development, conducted the laboratory work, performed analyses and visualization, wrote the original draft, and led subsequent editing with contributions from co-authors.

IV. Contributed to the study design, conducted the laboratory work, performed formal analyses and visualization, wrote the original draft, and led subsequent editing with input from co-authors.

List of figures

1. Background

When climate change was first conceptualized over two centuries ago, little attention was given to the potential impact of rising atmospheric temperatures on living organisms (Arrhenius, 1896; Edelsparre et al., 2024). Over time, research has clearly demonstrated that the effects of climate change on life on Earth are substantial, ongoing, and expected to persist throughout the next century and beyond (Edelsparre et al., 2024; Lyon et al., 2022). The most significant force generating such effects is global warming (IPCC, 2018). Due to anthropogenic activity, the rate of warming is at least ten times faster than any other climate shift that has occurred in the past 50 million years (IPCC, 2019; Sage, 2020). Projections suggest that the average increase in global surface temperature by 2100 relative to 1990 is unlikely to be contained under 2 °C (Raftery et al., 2017), with significant regional variability in frequency, intensity, and magnitude (IPCC, 2019). The consequences of such warming have been documented for both natural and anthropogenic systems, including all sorts of ecosystems (Sarmiento et al., 2004).

Temperature, the most critical abiotic factor governing ectotherms activity and life history – has pervasive impacts on these organisms (Angilletta et al., 2002; Reynolds & Casterlin, 1980). Due to the fact that most ectothermic organisms rely solely on the external thermal environment to regulate their body temperature (Angilletta et al., 2002) and the thermal dependence in their physiological processes (Bennett, 1984; Huey & Kingsolver, 1989), the impact of rising temperatures is unavoidable for ectotherms (Angilletta et al., 2002). Rising temperatures can exceed the physical and physiological scopes of organisms and further threaten their population viability, and thus have been associated with the accelerated extinction rates in the Anthropocene (Bestion et al., 2015; Massot et al., 2008; Urban, 2015; Zeh et al., 2012). Critical questions have arisen: will organisms have enough plasticity to expand their physiological and physical phenotype range? Will there be enough geographical refuge for species to survive? Will they have enough time to adapt, or will they succumb to these pressures and face extinction?

Ectothermic organisms constitute the vast majority of organism biomass and about 99% of species worldwide (E. O. Wilson, 2010). Within ectotherms, fish represent the largest and most diverse group of vertebrates,

with over 32,000 described species (Nelson et al., 2016). Fish play irreplaceable roles in food webs as both prey and predator, contributing to nutrient cycling, maintaining biodiversity and ecosystem functioning, providing vital social and economic services, and forming a significant part of our cultural heritage (Cowx & Portocarrero Aya, 2011; Holmlund & Hammer, 1999; Lynch et al., 2016; Tocher, 2003). In the face of exacerbating climate warming impacts, understanding the mechanisms behind ectotherms response to warming is crucial for predicting population dynamics and provide valuable insights on effective mitigation and conservation efforts in preserving biodiversity, maintaining ecological integrity and securing the services they provide to both nature and society.

1.1 Trait based biology in fish

The trait-based approach is a classic framework to tackle complex ecological questions (Andersen & Pedersen, 2009). Traits can be put into many different contexts, such as life history, fitness, function, physiology, morphology, behavior and performance (Dawson et al., 2021). Life history traits differ markedly among individuals, populations and species (Ricklefs & Wikelski, 2002). Metabolic rate is the most fundamental physiological trait: it reflects the energetic cost of living and is thought to set the pace of life (Auer et al., 2018). Metabolism represents both the processes of turning consumed resources into energy that can fuel other processes, as well as the synthesis of essential molecules (Fell, 1997). The metabolic theory of ecology predicts how metabolic rate controls biological and ecological processes, ranging from basic to complex, at all levels of organisation—from individuals to populations and ecosystems (Brown et al., 2004). It does so by setting the rates of resource uptake from the environment and regulating their allocation to survival, growth, and reproduction (Brown et al., 2004). Additionally, there is a general negative relationship between metabolic rate and life span (McCoy & Gillooly, 2008).

Fish typically follow an asymptotic growth curve, that is, the growth rate is exponential in the early stage of life and slows down at a later point in life when the fish become mature (Hopkins, 1992; Roff, 1993). Consequently, the body size is determined by the timing of maturation as those that mature early may remain relatively small for their age and vice versa (Roff, 1993). As maturation marks the shift in fish energy allocation from somatic growth

to reproductive investment which determines reproductive output and ultimately affects fish overall fitness, it is the most crucial life history transition (Dieckmann & Heino, 2007). While body size and growth can change through the life time of fish, maturation determines two definite stages: juveniles and adults (Dieckmann & Heino, 2007). Many studies use maturity-related traits, size- and age at maturity, and other proxies to study or infer maturation (Kendall et al., 2009; Trippel, 1995; Vitale et al., 2006). However, compared to maturity-related traits, maturation-related traits, i.e. the time point when fish become mature (which can be represented by the exact age or body size) are much more difficult to measure directly, as the time point can only be captured by continuously monitoring individuals throughout their life history. Evidently, this is only achievable in controlled environment where fish are in captivity (Dhillon & Fox, 2004; Stearns & Koella, 1986). Even so, the point of maturing can be influenced by the growth trajectory the individual employs, unless the growth rate is also controlled (Heino et al., 2002). To address this, estimating the probability of maturation as a function of size and age has been developed as a method to quantity fish maturation schedules, unveiling the true patterns of maturation masked by fluctuating growth rates and mortality (Heino et al., 2002).

Phenotypes are shaped by the interplay between genetic factors (some of which are heritable), environment influences (non-heritable), and their interaction (Kroon et al., 2025). Fitness is determined by how well the phenotype matches the environment, with natural selection acting on phenotypes and the underlying genetic variants that contribute to those phenotypes. Trade-offs are a core component within the realm of life-history evolution, balancing the fitness of two or more traits when a beneficial change in one trait is paid by the loss of fitness in another (Stearns, 1989). Without trade-offs, all traits would have been selected to maximum fitness, which is clearly not the case (Leimar, 2002). Reproduction is costly, requiring survival until maturation (age at first reproduction), and energy allocated to develop for fecundity (Bell, 1980). In fish, the cost of reproduction represents one of the most prominent life-history trade-offs in fish (Stearns, 1989). At a slower pace of life, a reduced growth rate can be a trade-off for higher fecundity, to reproduce more offspring with a longer lifespan and a larger body size. Conversely, with faster life histories, fish that mature early are typically smaller, but are able to reproduce earlier or more frequently (Kozlowski, 1996). The ecological and evolutionary change on

individual, population, community and ecosystem levels all boils down to basic biological traits (Pelletier et al., 2007) and trade-offs among them shape evolutionary trajectories by determining fitness under different environments (Roff & Fairbairn, 2007).

1.2 Trait changes in response to warming

An effective approach to investigate fish response to warming is by monitoring well-defined traits (Frimpong & Angermeier, 2010). Key traits that govern performance under different thermal environments (hereafter referred to as thermal performance traits) include upper and lower thermal limits, metabolic rate, and behavioural traits such as habitat choice and timing of activity (Neubauer & Andersen, 2019). Thermal performance traits differ largely between fish species; for example, the critical temperature at which fish can sustain basic activity can fall near 35°C, 25°C, 15°C or even 5°C depending on the species (Reynolds & Casterlin, 1980). Despite large variation in optimum temperatures of different fish species (Brett, 1956), rising temperatures have been consistently associated with decreased survival (Crozier et al., 2020) and increased mortality (Lindmark et al., 2023) in many fish species. Increased metabolic rate (Brown et al., 2004; Fry, 1957; Johansen & Jones, 2011) and thermal tolerance are found widely in fish and aquatic invertebrates (Healy et al., 2019). However, a compensatory metabolic response in the form of a depressed standard metabolic rate has been found in several warm-acclimatized or warm-adapted fish species (Pilakouta et al., 2020; Sandblom et al., 2016; Sylvestre et al., 2007), which can be a result of potential evolutionary adaptation in response to warming.

In fish, other widely recognized responses to rapid climate warming include shifts in (1) distribution and dispersal ranges (Comte et al., 2014; Parmesan & Yohe, 2003), for instance, many species are found to move poleward (Frainer et al., 2017); (2) phenology – the timing of life-history processes (Crozier & Hutchings, 2014), for example, advanced timing in reproductive season (Wedekind & Küng, 2010) and migration (Reed et al., 2011; Kovach et al., 2012; Otero et al., 2014) has been found in various species. In addition to these two, more and more studies refer to declines in body size as the third most common response to warming (Gardner et al., 2011; Sheridan & Bickford, 2011). This decline is closely linked to changes in vital physiological rates associated with thermal performance traits

(Neubauer & Andersen, 2019). Since body size is a key determinant of many critical aspects of fish life history, including energetics, predator susceptibility, species interactions and reproductive output, warming-induced changes in body size can in turn have very profound impact on fish population survival, size structure, and recruitment (Ahti et al., 2020).

The effects of warming vary across the life-history continuum and vary among individuals adopting different life history strategies (Grainger & Levine, 2022). Within a population, maturation of individuals typically occur at different ages and sizes as they follow different growth trajectories (Stearns & Koella, 1986). Likely associated with a faster developmental and growth rate at early life stages, a decrease in size at maturation has been found in fish under warming via direct observations following the life history of the fish or via model estimations (Dhillon & Fox, 2004; Kuparinen et al., 2011; Tobin & Wright, 2011). In contrast, a later age at maturity has also been observed (Otero et al., 2012). Many maturation studies employed the method termed probabilistic maturation reaction norms (PMRNs) by describing the covariation of age and size at maturation at a population level (Stearns & Koella, 1986). The benefits of PMRNs include: (1) less stringent data sampling requirements compared to continuous monitoring of fish life history; (2) the ability to account for plastic effects of growth, mortality, body condition, and other factors on maturation when data is available (Dieckmann & Heino, 2007). To date, however, studies implementing PMRNs rarely investigate effects of warming across multiple fish generations, particularly within a controlled experimental setup with consistent warming exposure. These studies have typically involved either scenarios with confounding factors in the wild or relatively short-term laboratory experiments with limited ecological realism (Niu et al., 2023). More importantly, if such a study can be carried out in a system with concurrent and consistent warming over time, insights into the evolutionary nature of maturation in response to warming can be gained, as the PMRNs method can account for the plastic effects of warming on age and size, revealing the underlying maturation response pattern, likely with a genetic basis (Hutchings, 2011).

The non-unidirectional responses to warming observed across various traits mentioned above may stem from the well-established hump-shaped relationship between temperature and thermal performance traits in ectotherms (Malusare et al., 2023), known as the thermal performance curve

(Figure 1). This relationship is evident in traits such as the metabolic rate in many fish species (Chen et al., 2015; Pilakouta et al., 2020; Schulte, 2015). Depending on the position of the temperature increase on the x-axis (the temperature axis), the performance at the new temperature may be on the same side or the opposite side of the hump compared to the performance at the original temperature (Figure 1). As a result, responses to warming in fish can vary depending on the specific temperature. Responses that involve species interactions, such as alterations in feeding rates, may therefore indirectly reflect the influence of such a thermal performance curve (Englund et al., 2011; Grigaltchik et al., 2012; Rall et al., 2012).

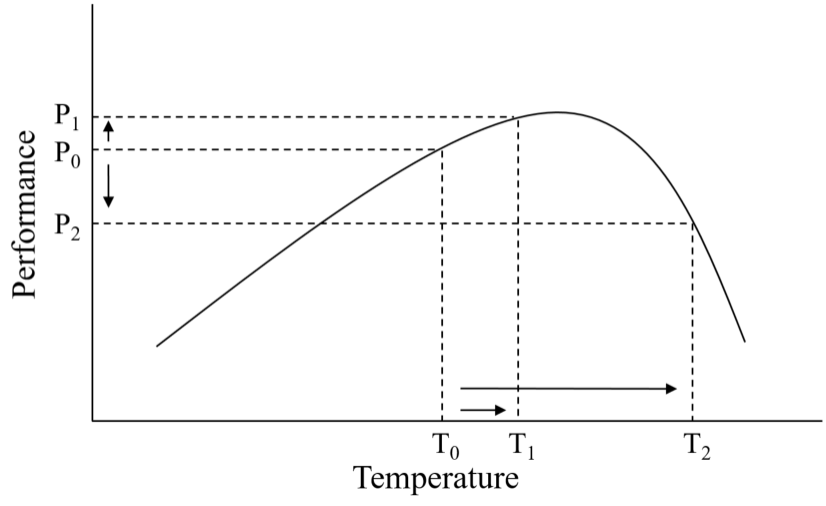

Figure 1. A hypothetical thermal performance curve of a fish, based on Huey & Stevenson (1979). The graph illustrates that although both new temperatures T_1 and T_2 are higher compared to the original temperature T_0, the corresponding performance can increase (P_1) or decrease (P_2) compared to the original P_0.

Many of these responses to warming can be attributed to plastic phenotypic changes (Crozier & Hutchings, 2014), or evolutionary genetic changes (Hoffmann & Sgrò, 2011) or both. In my thesis, I limit the scope of evolution to changes in allele frequencies in a population that can be passed on to the next generation (Endler, 1986). In the wild, natural selection occurs when a selection force acts on the various phenotypes of a trait in the population, by determining how well they fit to the current environment, and consequently, making a selection on the various genotypes underlying the

phenotypes. Genetic variation in a population is often maintained in heterogeneous or temporally variable environments, as it is intrinsically beneficial (Bell, 2010). Evolution occurs when such selection results in allele frequency changes in the population (Endler, 1986). In the context of warming, one must ask: how do the phenotypes fit the new thermal environment, and will the new temperature select certain underlying genotypes? Ultimately, will warming lead to evolutionary changes in fish populations over time? And if so, what molecular mechanisms drive the modification of fish phenotypes? To address these questions, using only the trait-based approach is insufficient to unravel the mechanistic pathways behind.

1.3 Warming-induced evolution in fish

> Nothing in biology makes sense except in the light of evolution (Dobzhansky, 1973).

Ever since the word ecology was coined, ecology has been intertwined with evolution (Mayr, 1982). Integrative understanding of biological processes keep being repeated and advocated in the literature (Johnson & Stinchcombe, 2007; Kokko & López-Sepulcre, 2007). The evolutionary and molecular perspectives to ecological integration is needed to advance the understanding of how climate warming will alter populations, species and communities (Lavergne et al., 2010; Pörtner et al., 2006). Warming can act as a selective pressure on existing genetic variation in a population, and, in turn, may shift allele frequencies by selecting phenotypic traits with a genetic basis (McGaughran et al., 2021). Adaptive evolution occurs when natural selection acts on heritable traits, resulting in genetic changes over generations. A key goal of evolutionary biology is to understand how populations adapt to novel conditions. The historical view of evolution is that it is relatively slow compared to environmental changes, however, more and more studies have shown that evolution can be rather rapid (Hendry et al., 2000; Reid et al., 2016; Rudman et al., 2022). Rapid adaptive evolution as a mechanism for facilitating species survival in novel environments might partially counteract the fast environmental changes due to climate change. Theory, experiments, simulations, and field studies all highlight the importance of evolutionary

potential - the capacity to change genetically that in turn increases fitness under changing conditions, in characterizing and mitigating extinction risk (Forester et al., 2022). Evidence indicating that fish have little evolutionary potential or, contrarily, that fish possess promising potential have both been collected (Barrett et al., 2011; Radchuk et al., 2019; Rummer & Munday, 2017; Sandblom et al., 2016). Moreover, it has been shown that rapid evolution induced by dramatic habitat changes caused by anthropogenic activities can lead to the loss of critical adaptive alleles (Prince et al., 2017). Such genetic diversity decline and loss will likely hinder current and future restoration efforts, as well as compromise resilience and evolutionary potential (Thompson et al., 2019).

Population genomics studies can identify particular genetic loci and variants responsible for responses to changing environments with a genetic basis, offering means for researchers to estimate the capacity of populations to evolve and adapt in novel environments (Hohenlohe et al., 2021). Historically, testing for evolutionary adaptation and dissecting its genetic basis required controlled breeding, common garden experiments or reciprocal transplant experiments, which are typically feasible only for a few model organisms (Matsuba et al., 2013; Schou et al., 2014). With the flourishment of the Next-Generation Sequencing (NGS) technologies over recent decades, whole genome sequencing (WGS), among other genetic tools, has been widely applied in non-model species to study their evolutionary responses to environmental changes (Giani et al., 2020). Certain genes and genetic markers influencing life history traits have now been identified, and WGS has been utilised to answer important ecological questions in some fish of particular interest (Ferrari et al., 2021; Östergren et al., 2021; Pinsky et al., 2021; Therkildsen et al., 2013; Uusi-Heikkilä et al., 2015; van Wijk et al., 2013).

In recent years, evidence shows that warming might be able to induce evolutionary changes in fish. For instance, signs of evolution linked to temperature have been found in wild fish populations inhabiting different thermal environments (Avaria-Llautureo et al., 2021; Bradbury et al., 2010; Chen et al., 2018; Jeffery et al., 2017; Narum et al., 2013; Pilakouta et al., 2020) and in laboratory studies where experimental temperature was manipulated across generations (Kavanagh et al., 2010; Loisel et al., 2019; Wootton et al., 2021). Modelling work simulating warming also suggests the existence of evolutionary responses to thermal selection (Crozier et al., 2011;

Reed et al., 2011). Genetic monitoring has been conducted in parallel with temperature variation records; however, such efforts are rare and have been restricted to microsatellite loci (Kovach et al., 2012). Notably, this approach successfully linked temperature changes to shifts towards earlier migration in a salmonid species (Kovach et al., 2012). Temperature also plays an important role in evolutionary responses to selection on other pressures such as parasitisim and over-fishing (Audzijonyte et al., 2016; Clark et al., 2017). Rising temperatures can amplify the effect of such stressors by increasing oxidative stress and osmoregulatory disturbance (da Costa et al., 2021; Dionne et al., 2007), or alleviate them by promoting faster growth rate and earlier maturation (Audzijonyte et al., 2016). Warming weakens the positive relationship between body size and fecundity, which relaxes the selection for highly reproductive individuals by size-selective fishing (Arendt, 2015).

In cases where warming-induced trait changes are evident, studies rarely demonstrate whether such modifications are caused by plastic responses, genetic adaptations, or some combination of the two (Bonnet et al., 2019; Merilä & Hendry, 2014). This is particularly true for wild populations under long-term warming exposure, where studying the appropriate spatial and temporal scales poses significant challenges. Additionally, confounding factors are often present in space-for-time substitution studies aimed to infer selection associated with temperature differences (Guo et al., 2015; Pilakouta et al., 2020). Despite extensive efforts in trait monitoring, the cellular and physiological pathways to fish responses to warming in complex traits, such as body size, growth, maturation and reproductive investment, remain largely unknown (Somero, 2012). A key limitation is the lack of comprehensive understanding of whether such traits in fish are genetically determined or the extent of their genetic makeup (Andersson & Georges, 2004; Naish & Hard, 2008; Waples et al., 2020), leaving critical gaps in our ability to conclusively demonstrate adaptive evolution in the wild (Olson-Manning et al., 2012). Overall, direct evidence linking warming and selection in natural fish populations remains limited. Due to the lack of knowledge on the role of warming-induced evolution, this fundamental biological process is still excluded from most predictive models of adaptive response to climate change (Urban et al., 2016), which can result in ineffective conservation effort and inadequate recovery planning (Forester et al., 2022).

1.4 Ecological consequences of the warming effects on fish

Nothing in evolution makes sense except in the light of ecology. Evolution does not occur in populations independently, as it acts on the interactions between individuals and their environments (Levins, 1968). Interactions among individuals, trophic levels and communities play a key role in evolution (Lawrence et al., 2012; Otto & Nuismer, 2004). Without considering species interactions, the overview of warming impacts on the ecosystem would remain partial and mitigation efforts to address the biodiversity crisis induced by climate warming would fall inadequate (Åkesson et al., 2021). The most common type of between-species interaction is the consumer-resource or predator-prey interaction (Barbosa & Castellanos, 2005). This interaction is considered a main driving force of population and community dynamics and the foundation of every food web (Polis & Strong, 1996; Polis & Winemiller, 1996; Ruiter et al., 2005). Small changes in phenotypic traits or shifts in population structures, can get either amplified or dampened through the intricate relationships of the food webs (Audzijonyte et al., 2013). Especially for endothermic organisms at high trophic levels, warming-induced changes in trophic interactions within the food-webs may be more important than direct physiological effects of rising temperatures (Kirby & Beaugrand, 2009).

In aquatic systems, fish typically employ size-specific feeding (Dörner & Wagner, 2003) and can exert top-down control on their prey communities (Gliwicz, 1994; Northcote, 1988). Warming can affect the feeding outcomes of fish directly via changes in their attack rates (Grigaltchik et al., 2012) or indirectly through changes in their metabolic rate (Brown et al., 2004), activity level (Colchen et al., 2017), locomotion (Johansen & Jones, 2011; O'Steen & Bennett, 2003), and morphology (Rall et al., 2012; Romanuk et al., 2011) in fish. These changes, in turn, can affect prey fish's susceptibility to their predators (Wagner & Benndorf, 2007). Consequently, warming-induced changes in prey fish can affect predator fish populations. In addition, fish can modulate the effects of climate change on the energy transfer from the lower to the higher trophic level by disrupting the stability of energy flow within marine - terrestrial coupling through trophic interactions (Luczak et al., 2011). For example, the reduction in lipid-rich copepods due to warming-induced range shifts has resulted in a decline in sandeel recruitment and led to poor breeding success in seabirds (Frederiksen et al., 2013). Warming-

induced range expansion of a single species can disrupt the original trophic cascades in the ecosystem (Stewart et al., 2014). Evidently, predictions on how communities will respond to warming need to be based on interactions within or between food webs to be accurate.

Warming can also act on mixed competition-predation interactions, e.g. intraguild predation between a predator and its prey while they compete for resources (Polis et al., 1989). Intraguild predation often results from changes in diet over ontogeny and strongly affects community composition and dynamics (Polis et al., 1989). For instance, warming might increase competition between juvenile predators and their prey for the same resources due to reductions in energetic efficiency (O'Connor et al., 2009). The intensified competition can hinder the maturation of juvenile predators, leading to a decline in the number of adult predators. This decrease in predation due to fewer adult predators on the prey reinforces the intensified competition between juvenile predators and prey and can, consequently, lead to the collapse of a predator population (Thunell et al., 2021).

Warming can influence the ecological consequences of fish response via behavioural mechanisms, such as dispersal or habitat selection (Boulanger et al., 2022). Temperature heavily affects individual vertical space partitioning and, given the existence of intraspecific temperature preference (McKenzie et al., 2021), shifts in water temperatures can influence encounter rates between predator and prey (Kjesbu et al., 2014). Changes in fish's social behavior in response to warming can affect the success rate of their anti-predation behavior (Webster et al., 2007), as warming can cause a reduction in shoal cohesion (Bartolini et al., 2015).

Consequently, interactions between species and trophic levels can propagate the effects of warming-induced changes in fish to other individuals, populations, communities, and ecosystems. These interactions densely connect organisms on all levels (Hampe & Petit, 2005). Understanding the ecological consequences of warming-induced trait and genetic changes in species is crucial for comprehending how warming can alter the ecosystems and providing strategic ecosystem-based managements (Audzijonyte et al., 2016).

There is compelling evidence of linkages between evolution and ecology of species and populations (Kokko & López-Sepulcre, 2007) and research incorporating both will further improve our understanding on how populations and communities respond to changing environments (Lancaster

et al., 2017). In this thesis, I employed an integrative approach to investigate how multi-generational warming induce evolution in fish, including trait evolution and genetic changes, and how such evolution can impose ecological consequences on the food web. The thesis tackled the knowledge gaps with respect to understanding the mechanisms behind fish evolutionary responses to warming, in particular, long-term ecosystem-warming, as well as their ecological consequences.

2. Aim of the thesis

The overall goal of this thesis is to investigate whether multiple generations of ecosystem warming induce evolutionary changes in wild fish populations, and to examine how such evolutionary changes affect other ecosystem components. The focus is specifically on chronic, long-term warming rather than acute, short-term heatwaves and temperature fluctuations. Specifically, the thesis addresses the following questions:

- Does multi-generational ecosystem warming induce evolution in wild fish populations? And if so, how? Specifically, the following aspects were addressed in different papers:
  - Trait evolution: indications of phenotypic changes in response to warming that persist across generations, potentially indicating evolutionary shifts
    - Maturation and reproduction related trait (Paper I)
    - Feeding related trait (Paper II)
  - Selection signatures in the perch genome (Paper III and IV)
- How does such potential evolutionary changes in wild fish affect their prey and food webs? (Paper II)

These questions encompass both the ecological (population and community levels) and evolutionary (molecular and population levels) consequences of ecosystem warming in fish. My hypotheses for each question and corresponding paper were:

Paper I: Fish size- and age at maturation decrease due to warming and decrease even more over time under the exposure of warming. Simultaneously, reproductive investment increases.

Paper II: Warming affects how fish feed on their prey and can be represented by the differences in the prey community composition. I refrain from hypothesizing with directions of feeding-related traits, since predictions on changes due to warming-induced adaptations in fish can be of any direction, which has been discussed in section 1.3.

Paper III & IV: The method developed in Paper III will yield useful genomic data, and warming will be associated with some selection signatures in the fish genome.

3. Methods and Materials

A diverse set of methods were employed to address the research questions posed in this thesis, using Eurasian perch (*Perca fluviatilis*, hereafter perch, Figure 2) as the model species in all studies included here. Perch is one of the most common and widespread fish species in Europe and Asia and has now been introduced to other continents (Collette & Bănărescu, 2011). In northern Europe, they distribute primarily in freshwater lakes and ponds but also appear in abundance along the brackish Baltic Sea coast. It is one of the key predators in these aquatic food webs, as well as an important prey for other fish and birds (Thorpe, 1977). Perch typically mature between the ages of two and five years (Heibo & Magnhagen, 2005), and the number of perch generations over different periods of time were calculated accordingly in this thesis.

In this thesis, data handling and statistical analyses were carried out using specialized bioinformatics tools alongside R (version 4.3.3, R Core Team, 2024). The computationally intensive bioinformatics analyses were supported by the high-performance computing resources provided by UPPMAX.

Figure 2. Photo by Mark Harris showing two relatively small Eurasian perch in an aquarium.

3.1 Ecosystem warming experiment (Paper I, II, III & IV)

My thesis utilised a semi-natural study system with a whole-ecosystem warming experimental setup. Located on the western Baltic Sea coast, the system consists of a chronically and artificially heated enclosed coastal area of ~1 km^2 and its adjacent unheated control area (Figure 3, also see Paper I, Figure S1; Paper II, Figure 1a). The enclosure was constructed in 1977 in order to monitor the effect of warm water effluents from the nearby nuclear power plant on fish populations and their surrounding environment (Thoresson, 1996). This design ensured that the perch populations from either the heated or unheated area were exposed to otherwise similar environmental conditions (Paper II, Table S1). Since 1980, the enclosure has been receiving warm water discharge (flow rate of 80-100 m^3/s), making its water temperature 5-10 °C higher than in the adjacent control area (Paper I, Figure S1). A metal mesh at the outlet (red arrow in paper I, Figure S1) acted as the physical barrier to the exchange of organisms (> 10 cm) until its removal in 2004. This resulted in a 23-year isolation of perch between the two areas, equal to 5-11 perch generations.

Figure 3. Aerial photo taken by Göran Hansson showing the constructed enclosure, often referred to as the heated area in this thesis.

As the most abundant fish species in both areas, perch has been monitored and studied most extensively over time (Adill et al., 2013). In the heated area: perch experience a higher mortality (Lindmark et al., 2023) and exhibit faster juvenile growth (Huss et al., 2019; Lindmark et al., 2023). However, for larger perch (size > 10 cm, age > 2 years), only females responded to warming positively in growth while males responded negatively (van Dorst et al., 2023). The spawning season starts earlier for female perch in the heated area than for perch in the unheated area (Lukšienė et al., 2000). When experimentally exposed to acute warming in a laboratory, large perch from the heated area displayed a lower metabolic rate, and a higher tolerance to high temperature (Sandblom et al., 2016). Perch from the heated area also have a higher mitochondrial gene expression in cardiac tissues (Pichaud et al., 2020) and a higher resistance to parasites (Mateos-Gonzalez et al., 2015). Collectively, this evidence suggests that the perch in the heated and unheated area have diverged into distinct populations.

To the best of my knowledge, only two studies have investigated the genetic differentiation between the perch populations from the two areas, both using very limited numbers of microsatellite loci. Demandt (2010) observed genetic differentiation between perch populations from the heated and unheated areas following the isolation of the heated population. While Björklund et al. (2015) reported allelic composition shifts in the major histocompatibility complex (MHC) in the heated-area perch population in relation to host-parasite interactions, possibly influenced by warming.

The temperature difference between the heated and unheated areas is large when compared to the predicted levels of warming in the Baltic Sea, as well as the global average temperature increase projected for the end of the 21st century (IPCC, 2018). This difference effectively creates a natural experimental setup with distinct heated and control treatments. For paper I, I used long-term monitoring data of perch collected from this system to track changes in key trait over time. In paper II, I collected egg strands from the system to hatch larvae, which were introduced into a mesocosm experiment. These larvae represent descendants of the heated and unheated populations – carrying the thermal history of perch from these two areas. All biological material used in paper III and IV was also collected from this system over the period of four decades.

3.1.1 Long-term monitoring data (Paper I)

To examine trait changes over the past four decades of warming, I exploited long-term monitoring data on focal life history traits of perch in the system. This dataset contains perch individual age, size, growth, population demography and dynamics dating back to the 1970s. Depth stratified multi-mesh gillnet fishing has been conducted since 1977 in both the heated and the reference area as part of a long-term monitoring program (Thoresson, 1996). The fishing was designed to cover the heated area and provided a similar scale of sampling in the unheated area, capturing the full size range of perch (Thoresson, 1996).

In paper I, I used data collected from all year-round fishing during 1983-2003, from which 3156 female and 3135 male perch were sampled in the heated area, and 1693 females and 2448 males in the unheated area. I used measurements of body size at capture, age, gonad weight, maturity status and total body weight. Sex and maturity stages were determined by macroscopic examination. All individuals included in my analyses were measured using the same standard and practice.

Age was determined by examining the annual rings on the operculum bone or otolith by experienced technicians. The operculum and otolith show allometric growth in relation to the fish body length, making it feasible to measure distances between yearly rings to estimate annual growth of perch and other fish species (Le Cren, 1947; Menon, 1950). Some derived parameters were calculated: (1) back-calculated growth trajectories: size at each age based on measurements of growth ring distances on the operculum bone, and (2) gonado-somatic index (GSI), defined as the percentage of gonad weight relative to total body weight (Rizzo and Bazzoli 2020), which was used as an indicator for perch reproductive investment.

In paper I, I adhere to the assumption that maturation is a deterministic process; that is, the onset of sexual maturity is fully determined by age and size of an individual. Probabilistic maturation reaction norms (PMRNs) estimate the probability of an individual maturing at a given age as a function of body size (Dieckmann & Heino, 2007). The reaction norms for age and size at maturation can be visualized as the size at which maturation occurs as a function of age (Stearns & Koella, 1986). This approach is commonly used when individual age and size at maturation cannot be observed directly. This is usually the case in fishery sampling data, where only a description of maturity status is recorded (Dieckmann & Heino, 2007). For example, it is

easy to estimate the percentage of mature fish in a population by sampling a subset of the population, but impossible to estimate the probability of a fish maturing at the same year by catching the fish. Since environmental factors can lead to variation in growth, there is also guaranteed variation in the maturation reaction norms, i.e. size and age at maturation (Gobin et al., 2021).

PMRNs can filter out the influence of varying growth and population structure on observed proportions of adults and juveniles at specific ages and sizes, distinguishing them from the actual process of maturation (Heino et al., 2002). This approach is the most commonly applied method for estimating PMRNs in the wild (Heino et al., 2015) and has been used for numerous fish stocks (Devine et al., 2012), such as northern cod (Olsen et al., 2004) and North Sea plaice (Grift et al., 2007). It has been used to identify and link decreases in age- and size at maturation due to decades of intense harvest in multiple species (Hunter et al., 2015). Most importantly, PMRNs have made it possible to strip away plastic effects from not only growth and mortality, but also factors such as, fish body condition and resource availability (Grift et al., 2007). As a result, shifts in the reaction norms — changes in age and size at maturation — are considered to have a genetic basis (Hutchings, 2011). However, many studies using PMRNs have not accounted for such plastic effects or explored the effects of environmental variables on fish maturation, largely due to the typical unavailability of such data (Uusi-Heikkilä et al., 2011).

I estimated the fish age- and size at maturation using PMRNs to investigate individual maturation schedules, including size at maturation for a given age, and by comparing the heated and unheated populations, I evaluated how warming has affected the maturation reaction norms independently from changes in maturity resulting from changes in growth or mortality. I derived PMRNs for each individual using its size at capture, age, and growth (back-calculated size at age) and the maturity ogive model (details see Paper I, Equation 1) to predict its corresponding maturity status following Barot et al. (2004). I then calculated the probabilities of an individual becoming mature at a given age and size using the maturity ogives of fish at a given age and size in the year of capture and the previous year, respectively, and the individual growth increment in between.

To compare perch PMRNs between the heated and unheated areas over time, I focused the analyses solely on two- to five-year-old females from two

groups of cohorts: 1980-1984 and 1991-1996. I estimated individual size-at-age from individual growth data (see paper I supplement for details), instead of fitting von Bertalanffy's growth curves to calculate a population average, a popular practice in the field of PMRNs studies. The benefit of using individual growth trajectories rather than a population mean is that I could calculate the individual probability of maturing at any given size or age, rather than a population-level average reaction norm. This revealed PMRNs variation among individuals in addition to changes in the population mean.

To investigate whether warming influences fish age- and size at maturation, and if so, how, I modelled the probability of maturing as a function of area (heated or unheated), time period (early: five-year warming or late: after multi-generation warming to include the potential effect of duration of warming), fish age (2-5), and size using binomial generalized linear models (GLMs). Pairwise differences in maturation probabilities between the heated and natural temperature area and time periods were assessed using the non-parametric Mann-Whitney U test. Similarly, I modelled GSI as a function of area, time period, fish age (3-5), size, and their interactions. Student's t-test was used to assess differences in GSI between the areas or periods.

For both maturation probability and reproductive investment GSI, I ran the null model and full model and every model in between them (see Paper I, Table S8 & S9). I selected the models manually through a backwards stepwise process, starting with the full model. The best-fitting model was determined on the lowest Akaike Information Criterion (AIC, Burnham & Anderson, 2002).

3.1.2 Mesocosm experiment (Paper II)

To study the ecological consequences of warming-induced evolution in fish on a food-web module, I conducted a common garden experiment of 38 mesocosms from May to July 2021 (Figure 4). I studied whether the thermal origin (heated or unheated) of larval fish affects their zooplankton prey communities and if so, how the effects vary under a temperature gradient.

Figure 4. The outdoor mesocosm experiment setup at the field site. In the blue plastic bags, I constructed 38 mesocosms. The visible black cords and white tubing going into the mesocosms were connected to the heaters (turned on in 29 mesocosms and turned off in the other 9) and provided oxygen to the mesocosms from the air pumps (not visible). In the light blue pools in front of the building, situated the 9 mesocosms that had their heaters turned off. The pool had a live flow-through of water from the unheated area, which acted as a cooling system for the 9 mesocosms. Together, "heating" and "cooling" manipulated the water temperature in the mesocosms and achieved a temperature gradient. In the building behind the pools, I hatched perch larvae from egg strands (Figure 5) collected from the heated and unheated area, i.e. larvae of heated and unheated origin.

I used fish larvae originating from the two adjacent wild populations (as described in 3.1 Ecosystem warming experiment): one unheated-origin population and the other heated for many generations (heated-origin population). Of the 38 mesocosms used in the experiment, 26 were inoculated with 10 fish larvae each (13 tanks with heated-origin and 13 with unheated-origin fish larvae) and the remaining 12 tanks were kept without fish as control (see paper II, Figure 1). During the experiment, fish larvae of both origins fed on the same zooplankton community from the unheated area. Both predators and prey were exposed to a gradient of experimental temperatures.

To assess the effect of fish origin and temperature, I collected samples from all the trophic levels: zooplankton and chlorophyll *a* on seven

occasions. I focused on three key time points for subsequent analyses: (1) prior to the addition of fish larvae to the mesocosms, (2) at the midpoint of the experiment and (3) approaching the end of the experiment. I identified, counted and calculated the abundance, biomass and composition of the zooplankton community.

I sampled the perch larvae at the end of the experiment, by a handmade drop net. Fish larvae were euthanized upon capture and transferred to storage in 80% ethanol. I counted the number of fish caught and measured their body size and weight to estimate fish survival and growth.

Figure 5. Left: one example of the collected perch egg strands (no eyes were developed yet) afloat in the water with the help of a mesh; right: about week-old hatched larvae in an aquarium.

3.1.3 The operculum bone archive (Paper III & IV)

The Department of Aquatic Resources, Swedish University of Agricultural Sciences (SLU Aqua), holds, like many fisheries institutes, archives of dried fish tissue samples, dating back to more than 100 years ago. An operculum archive was established in 1970 alongside the annual gillnet fishing program (see Long-term monitoring data). Depending on specific sampling procedures and species, different tissues (otoliths, opercula, scales, muscle,

etc.) have been collected. Eurasian perch is the species that has been most extensively monitored and sampled (Thoresson, 1996). Consequently, the operculum bone (Paper III, Figure 1) archive is the most abundant in terms of number and location, while also offering the highest temporal resolution. In particular, these archival bones were deliberately stripped of DNA-rich tissue by soaking them in boiling water followed by rubbing and cleaning. The age and growth trajectory used in Paper I were derived from these bones by measuring their annual growth rings by experienced technicians. Beyond providing information about fish age and growth, however, these thousands of operculum bones also preserve valuable genomic information of these individuals.

To investigate whether warming-driven natural selection has occurred in the perch populations, I analyzed the genomic data from the heated and unheated perch populations across three time points over four decades - from before heating started to after up to 20 generations of warming.

DNA was extracted from archival bones collected at two time points: 1977-1978 and 2001-2002. To evaluate whether (1) the storage time of the archived bones, (2) the type of tissue (bone or muscle), and (3) the treatments (e.g. boiling) and storage conditions of the bones influenced DNA yield and genomic data quality, I complemented the bone samples with fresh muscle tissue samples collected in 2021-2022 and conducted a comparative analysis of the samples DNA integrities and sequencing metrics. The addition of fresh muscle samples also allowed me to track the effect of 20 extra years, or approximately four to ten perch generations, of warming exposure on perch.

For simplicity, the archival bones collected from the heated and unheated populations were labelled as the 1980s bones (collected in 1977-1978) and the 2000s bones (collected in 2001-2002), with their respective populations named Warm1, Cold1, Warm2, and Cold2. Fresh muscle samples collected in 2021-2022 were labelled the 2020s muscles and populations: Warm3 and Cold3. This framework allowed for a comprehensive comparison of historical and contemporary genomic data to assess the evolutionary impact of warming on these populations.

3.1.4 Molecular analyses (Paper II, III & IV)

Microsatellite DNA

In addition to whole genome sequencing data generated and analysed in Paper III and Paper IV, I used microsatellite markers to genotype fish populations inoculated in the mesocosm experiment described in paper II. This genotyping aimed to provide an overview of population differentiation between the perch populations from the heated and unheated ecosystems and to explore how potential evolutionary changes induced by warming can affect fish prey. To achieve this, I sampled three individuals (larvae or eggs) from each of the 30 perch roe strands collected in the mesocosm experiment (Paper II). Each individual was genotyped using 14 microsatellite loci developed for perch (Paper II, Supplementary materials)

Genomic DNA extraction and validation

In Paper III, I used a commercially available DNA extraction kit for blood and tissue samples to extract DNA from archival perch operculum bones that have storage times of 25-50 years, addressing the challenges associated with these underutilised archival samples. Historically, such samples are challenging to work with due to the long-term storage and DNA degrading treatments they receive during archiving. These factors have led to their underuse in genomic studies despite their potential to reveal valuable insights into long-term evolutionary and ecological changes (Wandeler et al., 2007).

I optimized the DNA extraction process based on the standard manufacturer's protocol (NucleoSpin Tissue kit, Macherey-Nagel #740952.250). The important but minor adjustments are detailed in Paper III. In short, I initiated the tissue homogenizing process with bone pulverisation using high-speed bead beating. During the digestion process, I increased the amount of reagents and time at multiple steps. To minimize impurities and separate the digested sample from bone residue and beads, I added extra steps of centrifuging and transferring the sample supernatant to new sterile tubes. To acquire the DNA bound to the silica membrane as much as possible and preferably as high DNA concentration as possible, I lowered the total amount of elution buffer used, increased the buffer temperature, and added more time for incubation.

To verify the isolation of perch endogenous genomic DNA, I conducted end-point PCR using two microsatellite primers designed for perch. I measured the DNA concentration and assessed fragment size and

distributions. DNA quality was further assessed by comparing the total yield, fragment size and post-mortem damage between archived bones of different storage times as well as between archived bone samples and fresh muscle samples.

Whole genome sequencing (WGS)

Analyses on genomic data can reveal signatures of adaptive genetic variation, and by relating these signatures to environmental factors, adaptation can be linked to natural selection driven by those factors (Lotterhos & Whitlock, 2015; Savolainen et al., 2013). To test whether the DNA extracted from perch operculum bones can yield high-quality WGS data and subsequently study whether warming has induced natural selection using the genomic data, I submitted 231 perch DNA samples with a concentration exceeding 0.6 ng/µl for library preparation and short-read whole genome sequencing.

3.1.5 Population genetics and bioinformatics (Paper II, III & IV)

To assess the genetic differentiation between the heated- and unheated-originated perch larvae from Paper II, I conducted Fisher's exact probability test via Genepop version 4.7.5 using the 14 microsatellite DNA loci genotype.

For the whole genome sequencing data obtained from archived operculum bones and fresh muscle tissue (Paper III & IV), quality control was performed as the initial step. Read quality was assessed using FastQC/0.11.9 (Andrews, 2019) and sequences were trimmed to remove adapters using fastp/0.23.4 (Chen et al., 2018). The filtered reads were then mapped to the Eurasian perch reference genome (NCBI: GCA_010015445.1) using bowtie2 (Langmead & Salzberg, 2012), while mapped reads were processed and analysed in terms of genome coverage and depth using SAMtools/1.16 (H. Li et al., 2009). To specifically compare the effect of post-mortem DNA damage on read quality between the archival samples of different storage times and fresh samples, nucleotide substitution rates due to deamination were estimated with Mapdamage2.0 (Jónsson et al., 2013).

For single nucleotide variant (single nucleotide polymorphisms, SNPs) calling, I carried out GATK best practice pipeline version 4.3.0.0 (Auwera & O'Connor, 2020) on nuclear DNA (nDNA) and mitochondrial DNA

(mtDNA) sequences separately. Specifically, I used the HaplotypeCaller, CombineGVCFs and GenotypeGVCFs subroutine. I employed two versions of filtering on this initial SNP set (with 12,775,282 SNPs) using vcftools v0.1.16 (Danecek et al., 2011), details presented in paper IV and its supplement.

I assessed the level of cross-sample contamination by calculating the observed heterozygosity of mtDNA (mtDNA H_O) and the nuclear genome (nDNA H_O) using PLINK v1.90b4.9 (Chang et al., 2015). By examining the distribution of individual heterozygosity per population per time point, I determined the potentially problematic samples exhibiting excess or deficiency of heterozygosity and removed them for further analyses.

To evaluate genetic differentiation, I calculated the fixation index F_{ST} (Weir & Cockerham, 1984) for all pairwise population comparisons using the StAMPP R package (Pembleton, 2013). Nucleotide diversity (π) was evaluated at each site with vcftools. Principal components analysis (PCA) implemented in PLINK v1.90b4.9 (Chang et al., 2015) was used to explore clustering patterns and relationships among individuals based on their genetic variation. Population structure was further investigated using ADMIXTURE v1.3.0 (Alexander et al., 2009) to infer individual ancestry. Relatedness by descent was estimated using PLINK v1.90b4.9 (Chang et al., 2015). Gene flow between the heated and unheated areas was estimated using BayesAss (BA3 v3.0.5.6, Wilson & Rannala, 2003).

To identify outlier SNPs indicative of selection driven by warming, I used a consensus approach combining two methods: pcadapt (R package v4.3.3, Luu et al., 2017) and OutFLANK (v0.2, Whitlock & Lotterhos, 2015). To minimize potential biases from uneven numbers of females of males in the sampled populations, I excluded the sex chromosome, chromosome 18 (Kuhl et al., 2023) from this analysis. Putative outliers were identified in pairwise and three-way population comparisons: Warm1×Warm2, Warm2×Warm3, Warm1×Warm3, Cold1×Cold2, Cold2×Cold3, Cold1×Cold3, Warm1×Cold1, Warm2×Cold2, Warm3×Cold3, Warm1×Warm2×Warm3 and Cold1×Cold 2×Cold3.

I annotated the SNP functional categories using snpEff v5.2c (Cingolani et al., 2012) and the Eurasian perch annotation file (NCBI: GCA_010015445.1). For Gene Ontology (GO) analysis, I first identified perch genes orthologous to human genes and conducted a GO enrichment

analysis using R package GOSemSim (Yu et al., 2010) to identify overrepresented GO terms among the putative genes under selection.

3.2 Ethical considerations

The mesocosm experiment was conducted in accordance with national regulations for animal care, and the experimental design and practices were reviewed and approved by the regional review board for ethical animal experiments in Uppsala, Sweden. Approved permit no.: Dnr 5.8.18-04546-2021. Fish were removed from the mesocosms and euthanized in a benzocaine solution at the end of the experiment. All staff involved have completed animal ethics training.

4. Results and Discussion

In this thesis, I show that ecosystem warming has significant and substantial effects on fish individuals and populations, both phenotypically (paper I) and genetically (paper IV). My findings demonstrate that these warming-induced effects on fish can directly influence their prey community and, potentially, other trophic levels (paper II), indicating profound ecological consequences for the ecosystem.

4.1 Fish phenotypic responses to warming (Paper I & II)

In Paper I, I found that warming affected fish size at maturation and reproductive investment in the wild and the effects of warming differed over time. Initially (after the first several generations of warming exposure), perch in the heated area had a higher probability of maturing at all sizes between the ages of two and five years, which can be interpreted in two ways: (1) perch mature at a smaller size at these ages; (2) perch mature at a younger age at a certain size. The heated population also invested more energy in reproduction compared to perch in the unheated population. After four to eight generations of warming, the variation in size at maturation increased for perch in the heated area while the reproductive investment levels were similar in perch between the areas. Over time, the maturation reaction norms of perch in the heated area shifted. Because the method accounted for the plastic effects of growth and mortality, this shift indicates that warming may have induced evolutionary changes in maturation-related traits in a wild fish population, potential resulting from shifts in fish ontogeny and life history strategy.

In the context of evolution – to maximize fitness – a smaller size at maturation may be beneficial under warming conditions. This is because a smaller adult size allows more resources and energy to be allocated to reproductive development rather than somatic growth and maintenance. This is particularly advantageous at elevated temperatures as maintenance costs are often higher (Forster et al., 2012). A younger age at maturation (at a given size), on the other hand, can translate to more reproductive seasons given a stable life span, thereby increasing reproductive success in unpredictable environments or compensating for potential early mortality (Slatkin, 1974). This aligns with observations of increased mortality in perch from the heated

area (Lindmark et al., 2023). After multiple generations of warming, the variation in size at maturation increased. This may be attributed to unaccounted plasticity or suggests that some individuals in the population employed an alternative life history strategy: delayed maturation at a larger size (Stearns, 2000). It is beneficial to generate offspring to employ different life history strategies and display a variety of phenotypes in fluctuating and unpredictable environments, so that some will survive as the risk is spread among all offspring, which is referred to as bet-hedging (Pires et al., 2023; Slatkin, 1974).

I would like to discuss some limitations in Paper I so that readers can carefully interpret the results. (1) The sample size was somewhat smaller than what would be ideal for the PMRNs approach (Barot et al., 2004). This is a common limitation and especially challenging when sampling wild fish populations. (2) Due to a lack of body weight data, I could not incorporate fish body condition in the analyses. Including body condition might improve the estimation of the maturation schedule, as it could capture additional environmental variation (Grift et al., 2007; Vainikka et al., 2009). The more plasticity introduced by biotic or abiotic factors is accounted for, the more accurate and realistic maturation reaction norm estimations may become (Diaz Pauli & Heino, 2013). However, in addition to temperature, plastic effects on maturation from factors such as resource availability (Uusi-Heikkilä et al., 2011), population density growth (Kraak, 2007), social environment (Diaz Pauli & Heino, 2013), and habitat characteristics (Morita et al., 2009) were not considered in Paper I. (3) I chose identical sampling weeks for the GSI analysis. However, warming is known to shift spawning timing in fish (Miranda et al., 2013), and such shifts have been observed in our study system (Lukšienė & Sandström, 1994, Paper I, Figure S2). Therefore, controlling for sampling weeks may have resulted in sampling perch closer to spawning in the heated area than in the unheated area. This could obscure the true pattern behind the temporal dynamic of gonad development between perch from the two areas. (4) The analyses were based solely on data from female fish, leaving knowledge gaps regarding how temperature affects maturation schedules in males. Male fish may exhibit alternative reproductive tactics that warrant further exploration (Oliveira et al., 2008). Despite these constraints, the main findings in Paper I suggest that warming indeed alters the maturation timing and reproductive investment in a wild fish population, providing concrete evidence on warming-induced

responses in another important life history trait in this study system, as well as valuable insights into how ectotherms may respond to warming.

From Paper I, I have identified that maturation as a key trait that can be directly impacted by ecosystem warming. The PMRNs method is reliably filtering out influences of ecological changes on body growth and mortality, hence the shifts in maturation reaction norms indicate evolutionary changes (Heino & Dieckmann, 2008). However, the biggest caveat of PMRNs is that it cannot completely disentangle phenotypic plasticity from genetic change governing these traits in the absence of genetic data (Heino & Dieckmann, 2008). Whether such shifts in maturation reaction norms are evolutionary or not cannot be concluded, but at best, inferred. Therefore, investigating the genetic basis underlying these long-term phenotypic trait changes is necessary. A genome-wide screen of footprints of selection of perch from the heated and unheated areas would be a potential step to determine if warming has exerted selection on genetic components related to maturation and reproduction.

4.2 Fish genetic responses to warming (Paper II, III & IV)

I demonstrated that archival operculum bones can yield sufficient DNA and high quality whole-genome sequencing data, thereby unlocking the vast genomic potential of such samples. Bones and muscles differed substantially in DNA yield (Paper III, Figure 3), however, between the 2000s bones and the 2020s muscles, surprisingly similar levels of fragmentation, degradation and cross-contamination level were observed from their DNA samples (Paper III, Figure 5, 6 & S2). Interestingly, the mtDNA Ho was higher in the fresh muscle samples compared to the 1980s bones. While I cannot provide a definitive explanation, I suspect this may be related to nuclear sequences of mitochondrial origin (NUMTs) being incorrectly mapped as mtDNA (M. Li et al., 2012; Wei et al., 2020). However, it remains unclear why NUMTs would affect DNA from fresh muscle samples more significantly than DNA from the archival bones. The difference between mtDNA H_O and nDNA H_O patterns may suggest that mtDNA may not be a reliable indicator for assessing cross-contamination in this particular case. Most importantly, the varying qualities in the extracted DNA did not have a major impact on the whole genome sequencing data's generally high quality. Overall, I achieved high coverage (> 90%) of the genome across all types of samples and a

greater than 9-fold sequencing depth (Paper III, Figure 4). These sequencing data allowed me to retrospectively track the genetic changes in wild fish populations over a 44-year period of warming (Paper IV). They also represent the first demonstration of whole genome sequencing data generated from boiled archival operculum bones of fish (Caccavo et al., 2024; Pinsky et al., 2021; Pukk et al., 2013).

The time-efficient and cost-effective protocol presented in Paper III requires only a few adjustments from the standard manual provided in the commercially available DNA extraction kit. Like many DNA extraction methods developed and published, this demonstration (in Paper III) may be specifically designed for one type of sample, namely perch operculum bones. However, it highlights the great potential of high-quality genomic information from such spatially and temporally abundant, yet underused, archival samples. Furthermore, the experiences from this example demonstrate that adjustments can be easily made to the protocol to accommodate other sample types.

4.2.1 Population genetic differentiation and characteristics

In Paper II, the genotypes of 45 heated- and 45 unheated-originated perch larvae on 14 microsatellite DNA loci showed statistically significant genetic differentiation at a low level (Fisher's exact probability test, $X^2 = 82.5$, $p < 0.001$, $F_{ST} = 0.006$). This was the only support indicating that the inoculated fish larvae were somewhat genetically distinct prior to conducting the mesocosm experiment. Previous studies in the same study system found similar levels of genetic differentiation between perch populations from the heated and unheated areas sampled at earlier time points (Björklund et al., 2015; Demandt, 2010).

The low but significant genetic differentiation between the perch populations in the heated and unheated areas was further confirmed by the WGS analysis (Paper IV, Figure 2a). However, whether this differentiation is indicative of multi-generational warming-induced evolution remains unresolved. Furthermore, larger census sizes in these populations could buffer genetic changes, slowing the pace of differentiation. Establishing causation requires more robust evidence, which is why the genomic scan conducted in Paper IV was essential.

4.2.2 Detection of selection signature

From a total of 874,686 SNPs across the whole genome, I identified 1,573 candidate SNPs exhibiting signs of natural selection potentially induced by warming. The most significant candidate genes and gene families were the cadherin family (CDH) and the solute carrier families (SLC). The most enriched gene ontology (GO) terms were ion transport, synaptic activity, and neuron function. Notably, water temperature influences the activity of various neurotransmitters (Alfonso et al., 2021) and warming can induce physiological stress responses in fish via two main neuroendocrine pathways: the hypothalamic-pituitary-interrenal axis, and the brain–sympathetic–chromaffin cell axis (Alfonso et al., 2021). Neurotoxic effects of warming have also been observed in fish (Beltrán et al., 2021; Maffioli et al., 2023). These combined effects may explain the enrichment of GO terms related to synaptic and neuron functions. Neural processing is also metabolically expensive, with the brain consuming a significant portion of a fish's energy to maintain ionic gradients and electrical activity (Soengas & Aldegunde, 2002). One strategy to deal with increased energetic costs of the cellular stress response might be to conserve energy through altered metabolism. This may align with the selection signature observed in fatty acid-binding protein (FABP) genes (Paper IV), which regulate metabolic processes (Tocher, 2003). In addition, warming has been found to cause modifications to cell membranes and alterations in the intracellular environment that disrupt their structure and function in fish (Hazel, 1984; Little et al., 2020). Ion transport is critical for all cellular activities, particularly in excitable cells where membrane function relates to ion permeability (Alberts et al., 2002). This may explain why the three GO terms—ion transport, synaptic activity, and neuron function—were found to be enriched together.

By sampling fish populations at three time points over a 44-year period of artificial warming, I was able to investigate the genetic basis and architecture of contemporary evolution. The sampling began before the warming commenced. The evidence found in Paper IV suggests that natural selection has occurred in the system, and is likely associated with consistent warming. Given the existence of records for perch life history traits, such as body size and growth, conducting genome-wide association studies (GWAS, Uffelmann et al., 2021) is a logical and valuable next step to determine whether there are alleles at different loci underlying these significant

phenotypic variations observed in perch in the heated area. This approach has been employed in many studies linking genetic components to traits changes due to environmental influences (Gonzalez-Pena et al., 2016; Prchal et al., 2023).

In contrast to studies investigating functional annotation and candidate genes of temperature-associated outlier SNPs (Boulanger et al., 2022), I did not find any significant selection signatures in heat shock protein genes or heat tolerance related enrichment terms. This might be due to that my study design focused on the effects of chronic and constant warming rather than acute and extreme heat shock. Secondly, detecting polygenic traits using single-locus methods is challenging (Pinsky et al., 2021), yet these traits are likely the ones most affected by warming (Debes et al., 2021; Triantaphyllopoulos et al., 2020). Thirdly, Schierding et al. (2016) have suggested that variants in intergenic regions can have genome-wide effects as key modifiers of growth, acting through a regulatory network. For example, an intergenic locus was recently linked to growth in mandarin fish (*Siniperca chuatsi*, Liu et al., 2024). Single-locus variation in intergenic regions can appear to control complex traits via tightly linked blocks of genes (Oomen et al., 2020) or spatially linked co-inherited genomic region haploblocks due to linkage disequilibrium (Bartonicek et al., 2017; Brodie et al., 2016). The enrichment of candidate outlier SNPs found in intergenic regions (Paper IV) may be related to this. Additionally, SNPs with a selection signature are rarely found to be the causal variant for the associated trait (H. Li et al., 2016). In summary, further investigation is needed to better understand the enrichment and GO results.

During data exploration of genomic sequencing used in Paper IV, I have found signs of genetic markers other than SNPs, e.g. structural variants like chromosomal inversions. Chromosomal inversions can play a role in adaptation by maintaining locally beneficial haplotypes (Kirkpatrick & Barton, 2006) and copy number variations have been used to understand climate-related genotype-phenotype associations (Cayuela et al., 2022; Wellenreuther & Bernatchez, 2018). I have not fully exploited the temporal aspect of the allele frequency change of the selection signatures as I have only identified the outliers using different combinations of populations sampled at different time points. For example, Buffalo & Coop (2020) developed a method that separates the temporal covariance of allele frequency changes from the background signal of genetic drift. I am also

aware of the filtering routine termed “hard filtering” that is frequently used (Auwera & O’Connor, 2020), that differs in study-wide filtering as well as within-group filtering compared to what I used in Paper IV that can most definitely yield different results (see Supplement and Figure S8 in Paper IV), e.g. global MAF filtering (of different thresholds) can lead to the removal of critically informative, globally rare but locally common alleles, and insufficient filtering will leave sequencing artefact within the data. The effects of filtering are, in fact, an issue that requires greater attention yet remains largely overlooked (Hemstrom et al., 2024). Further investigations into these aspects might provide a more comprehensive picture of the genomic changes induced by warming.

In the future, genetic monitoring methods that provide comprehensive genomic data—beyond the relatively limited insights offered by a few mitochondrial DNA loci or microsatellite loci, or SNPs—should be prioritised. Integrating these approaches with traditional trait monitoring and analysing existing archived samples can enable retrospective tracking of evolutionary changes over time, extending our understanding of warming-induced evolution across both temporal and spatial dimensions. Future research should integrate multiple data sources, including comparative genomics, transcriptomics, proteomics, and epigenetics, alongside environmental datasets (Roscito et al., 2018). This approach should be complemented by targeted experiments designed to test hypotheses and uncover the mechanistic pathways that underlie evolutionary responses to warming.

4.3 Ecological consequences of warming-induced fish evolution (Paper II)

Results from the common garden experiment showed that, at the end of the experiment, there were more zooplankton prey remaining in the mesocosms with heated-origin larvae compared to those with unheated-origin larvae, both in total abundance and biomass. The differences in prey abundance and biomass varied with experimental temperatures and were most pronounced at higher temperatures (Paper II, Figure 2). The zooplankton community composition also differed between the two origins of their co-existed fish larvae and varied with experimental temperature (Paper II, Figure 3).

The observed effects of fish origin on zooplankton were likely due to differences in the feeding of perch larvae from the two origins, as fish survival and growth did not differ between the heated-origin and unheated-origin larvae (Paper II). One of the key results - that higher prey abundance remained in mesocosms with heated-originated larvae indicating reduced feeding levels or a preference for larger zooplankton - was not directly observed in their feeding behaviour. However, the controlled experimental conditions (especially the controlled zooplankton communities) strongly indicate that these differences likely resulted from variations in fish feeding strategies. This may have resulted from direct changes in fish feeding behaviours, such as a reduction in their attack rates (Grigaltchik et al., 2012; Sohlström et al., 2021), or from a decreased energy intake, likely due to a depressed metabolism in fish to compensate for the higher energy loss at higher temperatures (Pilakouta et al., 2020). The finding of lower abundance of large-sized zooplankton in mesocosms with larvae of heated origin suggests that these larvae may adopt a different life history strategy, characterized by a faster growth and development rate. This accelerated pace could enable them to predate on larger zooplankton earlier compared to larvae from the unheated origin. While the findings in Paper II did not conclusively demonstrate warming-induced alterations in fish feeding, they were sufficient to address the research question regarding the ecological consequences of warming effects on fish, as reflected by their prey community.

There are shortcomings of simulating an ecosystem in an experimental mesocosm (Paper II). For instance, a mesocosm as a partial miniature of the ecosystem cannot simulate important components like microclimate within a macroclimate (Woods et al., 2015). The limited experiment duration and water volume, and the simplification of the food web module implemented in the mesocosms may not represent the complex food webs in nature. Additionally, this study has focused solely on the larval stage of perch. Caution should be exercised when extrapolating findings from larvae to fish in other life stages (Nunn et al., 2012). Nonetheless, early life stages are crucial determinants of an individual's life history trajectory (Fuiman & Werner, 2009; Robert et al., 2023). Furthermore, an individual's thermal history significantly influences their thermal performance as an adult (Jonsson & Jonsson, 2014; Kellermann et al., 2017). Despite these limitations, the findings in Paper II offer valuable insights into how the

effects of warming at one trophic level can cascade down to another. A mesocosm experiment with a full-factorial design, namely incorporating plankton communities from the heated area in addition to the current experimental set-up described in Paper II, would provide additional insights into how long-term warming has affected diversity and evenness in zooplankton communities. Specifically, it could reveal whether warming has led to the dominance of a few taxa potentially adapted to warmer conditions (Thomas et al., 2012). More importantly, it can help answer whether the changes observed in the heated origin larvae were in fact adaptive to the responses in the zooplankton communities.

Although I did not investigate the secondary effect of changes in age- and size at maturation on the ecosystem in Paper I, several pathways could link these trait changes to ecological impacts on the population and community. For example, a decrease in maturation size could lead to a decline in mean adult size and fecundity in the population, which, in turn, may reduce the population recruitment capacity (Hutchings, 2002) and biomass production (van Dorst et al., 2019). However, previous research in the same study system as my thesis showed that adult size in perch is, on average, larger under warming (Lindmark et al., 2023). This, along with the greater variation found in age- and size at maturation after multi-generational warming (Paper I), points to the potential selection for an alternative life history strategy where some individuals might have adapted to mature at a later age and a larger adult size (Winemiller, 2005). Changes in body size can also cause shifts in predator–prey interactions and result in species diet changes that can further affect the overall food web stability (Lindmark et al., 2019; Thunell et al., 2021) and ecosystem functioning (Fisher et al., 2010).

Moreover, the effects of warming may interact with other external pressures such as extreme temperature events, parasitism, exploitation and eutrophication (Björklund et al., 2015; Dionne et al., 2007; Jane et al., 2024; van Dorst et al., 2019). The occurrence of heat waves is tightly linked to long-term warming trends (Oliver, 2019) and heat waves are likely to exacerbate the adverse effects of long-term warming (Woolway et al., 2021). In my thesis study system, the perch populations may have been exposed to increasing thermal fluctuations and severe heat waves, in addition to the long-term warming (Pichaud et al., 2020), which could potentially confound the findings of my thesis. Additionally, other often-neglected aspects, such

as the social structure within the fish population, have only recently been considered (Colchen et al., 2017), but may also influence the results.

Different life strategies as a result of warming responses could translate to interspecific or intraspecific niche separation (Pörtner et al., 2010). Individuals at the ends of the continuum may have distinct functional roles in predator-prey and ecosystem dynamics. For example, as the maximum body size determines the ecological niche a species occupies (Werner & Gilliam, 1984), individuals that mature early at a smaller size may occupy a different niche compared to those that mature later at a larger size (Paper I). From Paper II, warming might have caused fish larvae to partition into different trophic niches (feeding niche separation) where some feed on large-size prey and others on small-size prey to avoid competition for the same resources (Deary et al., 2017; Gladfelter & Johnson, 1983).

Finally, I would like to emphasise some key aspects of the study system in my thesis, so that readers can interpret the results with caution. Although it resembles an ecosystem experiment with manipulated temperature, it has no replicates. This limitation not only restricts the ability to examine variations in responses to warming across replicates but also raises concerns about the influence of other environmental or biological changes that may have specifically affected this system. For instance, over the years, the system has been stocked with eels and seals and has likely been subject to unauthorized fishing (Adill et al., 2013). How this may have affected the perch populations between the heated and unheated area is unknown and may not be feasible to investigate in the absence of available data. Additionally, the effects of continuous gillnet fishing as part of the long-term monitoring program are also unknown. The specifics of how exactly organisms can migrate in and out of the heated area are also ambiguous. During times when the grid at the heated area outlet was clogged, a reserve outlet was opened to release the effluent (Neuman and Sandström, 2002; Adill et al., 2013), thus, making it possible for fish to move between the two areas. Although results in Paper IV have showed low migration rates of perch between the two areas (Paper IV, Table S2), care should be taken when viewing this estimation derived from BayesAss analysis. Despite BayesAss being a method that is relatively free of assumptions, it can inflate gene flow estimates especially when the between-population F_{ST} is low (Cayuela et al., 2018; Meirmans, 2014), which was the case for the heated and unheated perch populations. Moreover, the unheated area is not free of the influence of warming as it is

still in the close vicinity of the warm water discharge. This might be especially true when the reserve outlet was opened up from time to time before the grid removal at the official outlet in 2004 (Adill et al., 2013). Nevertheless, this did not affect the general thermal differences between the two areas shown by their average water temperatures (Paper I, Figure S1c). The large difference in water temperature offered a unique opportunity to follow the history of warming, which is exceedingly rare and costly to conduct either in the laboratory or in the field.

Although I have employed a suite of approaches encompassing a broad range of biological methods, the studies were not sufficiently integrated to establish a direct link between changes in phenotypic traits (such as maturation and feeding) and genetic changes. This is largely due to a knowledge gap, both within the specific study system of my thesis and in the broader field, concerning the genetic underpinnings of body size, maturation, feeding, and other life-history traits in perch. This knowledge gap extends to most fish species, with the exception of a few high-value species (Andersson & Georges, 2004; Naish & Hard, 2008; Thorgaard et al., 2002; Waples et al., 2020). Compared to mammal and avian species, genomic resources of fish, including reference genome assemblies, are generally lacking (Houston et al., 2020). Furthermore, complex traits like body size and maturation are intrinsically difficult to investigate because they represent the combined effects of numerous interacting biological and ecological processes, acting as both causes and consequences that shape and influence other traits (Froese, 2022; Saborido-Rey & Kjesbu, 2005). Gaining knowledge of these fundamental aspects can improve the integration of ecological and evolutionary studies on how warming alters species, and their interactions with the environment, which helps us better understand and anticipate ecosystems' future responses to climate change.

5. Conclusions and Outlook

Using a combination of methods, my thesis provided concrete evidence that warming can drive evolutionary changes in fish and that these changes can have significant ecological impacts. By leveraging long-term monitoring data, field-based common garden experiments, and genomic analyses, findings in this thesis infer a feedback loop between ecology and evolution. Specifically, Paper I confirmed that warming causes ecological trait changes in fish; Paper IV revealed warming-induced evolutionary changes; and Paper II illustrated that these potentially evolutionarily adapted fish can influence organisms at another trophic level.

The findings in my thesis highlight the need for scientists and managers to reconsider the capacity of wild fish populations to adapt to, and recover from, rapid and intensive environmental changes. This series of studies, which focused on long-term ecosystem-scale warming, provides valuable insights for making more accurate predictions regarding the future dynamics of ectotherm populations, amid the challenges posed by global warming.

More broadly, this thesis demonstrates the importance and feasibility of considering evolutionary processes alongside ecological and demographic factors when assessing the total impact of warming. Understanding the interplay of these processes is essential for projecting population responses to warming. There remains a critical need to further synthesize the interaction between ecological and evolutionary responses to global change and also to deepen our understanding of the genetic basis underlying phenotypic adaptations.

However, any approach, whether empirical or theoretical, must acknowledge the limitations of made assumptions, significant uncertainties and issues of representativeness associated with what was specifically measured or simulated. Responses in one species or at one life stage can be difficult to interpret in relation to those in other species or stages; thus, conducting studies using only one species, as done in this thesis, cannot predict the potential effects of climate change on the abundance, distribution, and diversity of all species. However, it is currently virtually impossible to conduct detailed studies on every single species across all life stages. A practical alternative would be to start developing a general theory based on fundamental biological principles, which can then be utilised to make testable, quantitative predictions.

As the effects of climate change are expected to intensify (IPCC, 2022), the need for continued investigations on this topic cannot be overstated. What was once considered extreme warming may soon become the new normal (Woolway et al., 2021). The non-analogous nature of future climate scenarios further emphasizes the importance of using existing data for hindcasting to gather as much information as possible (Veloz et al., 2012). This thesis, along with other studies, demonstrates the potential for species and populations to adapt to warming. However, these heritable responses are unlikely to sufficiently counteract negative impacts from accelerating biodiversity loss and other associated challenges (Parmesan, 2006; Radchuk et al., 2019). Ongoing research, combined with effective conservation actions, is imperative to mitigate the challenges posed by warming.

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Popular science summary

Most people have probably heard or experienced one thing or another related to climate warming by now, be it another record-breaking hot summer day or the ever-melting glaciers. It does not, however, only affect humans, but profoundly impacts other living beings and the ecosystems we live in. As cold-blooded organisms that rely on their environment to regulate their body temperature, fish can be particularly vulnerable to warming waters. Many have heard of 'survival of the fittest'—the optimistic may find comfort in it, believing that at least some 'chosen' individuals will survive because they manage to 'evolve' alongside the novel temperatures. However, the extent of warming can be so extreme that it exceeds the limit of how organisms can possibly adjust, or it occurs so rapidly that there is not enough time for them to adapt and survive.

To ensure that fish thrive in their native environments and maintain their complex relationships with prey, predators, and competitors, we must understand how warming affects them. This knowledge is crucial for preserving Earth's remarkable biodiversity and preventing fish from becoming mere relics of our memories and historical photographs. We must ask, can fish evolve in response to rising temperatures? If so, to what extent and through what mechanisms? Do they adapt to become more heat-resistant? Can their offspring inherit this resistance, preparing them for an increasingly warm environment? Furthermore, is it possible for us to influence or reverse this potential evolutionary process?

Many scientists have addressed these questions and found that fish respond to warming in various ways, such as increased tolerance to higher temperatures, elevated metabolism, altered swimming behaviour, and changes in growth rates and final body sizes. For instance, some studies have shown that fish grow faster but reach a smaller adult size after being exposed to warmer conditions for multiple generations. These findings suggest a possible evolutionary component in the underlying mechanisms.

In this thesis, I studied the effects of long-term warming on fish evolution within a unique system consisting of two adjacent, similar coastal ecosystems. One of these ecosystems has been artificially heated and has maintained a temperature that is at least 5 ℃ warmer on average than the other since 1980. The heated area is a 1 km2 enclosed basin receiving cooling water discharge at a rate of up to 100 m3/s from the nearby Forsmark Nuclear

Power Plant. The enclosed area was constructed before the operation of the nuclear reactors, with the intention of monitoring the impacts of warming on fish and the ecosystem, allowing for comparison with the unheated ecosystem in the surrounding archipelago. This setup effectively creates an experimental system with one treatment (heating) and one control. Over time, it has served as a time machine, enabling generations of researchers, including myself, to retrospectively track the effects of warming on fish and their environment.

I used Eurasian perch, a species that is abundant in this system, as my model species for this thesis. Perch have been regularly sampled during a monitoring program conducted by the Department of Aquatic Resources at the Swedish University of Agricultural Sciences (SLU Aqua) within the study system. By analysing the long-term records of size, age and maturity status, I found that when perch mature (are able to reproduce for the first time), their body sizes are smaller, and their gonads (reproductive organs) are relatively larger in the heated area compared to perch from the unheated area. Fish that are smaller at the time of first reproduction, are likely to lay fewer eggs than larger fish; however, this may be offset by their more developed gonads. I also discovered that perch may start reproducing at a younger age, which could serve as a survival strategy in response to a potentially shorter lifespan due to warming. This pattern was observed only in the perch populations that had been exposed to warming for one or two generations. In contrast, perch in populations that have been exposed to warming for four to eight generations, exhibit a wider range of sizes when they mature and may not necessarily reproduce at a younger age or possess larger gonad. This change over time reflects a possible evolutionary component in perch's responses to warming.

To explore the genetic foundation of evolutionary responses to warming in fish, I extracted DNA—the genetic information that codes for the heritable traits and functions of organisms—from perch in the heated and unheated areas over time. I selected three time points (1980, 2000 and 2020) to track the past four decades of warming. For the first two time points, I extracted DNA from the operculum bones of perch, which are hard, flat flaps that cover their gills. These operculum bones were collected during the same monitoring program that generated records of perch size, age and maturity status. By comparing the genetic information between perch from the two ecosystems over time, I identified signals of natural selection due to

warming. However, the precise connections to specific traits in perch, such as how their maturation and reproductive processes, remain to be established.

To assess the secondary effects of warming on the food web through its impacts on fish, I created a miniature coastal ecosystem in 38 tanks. For three weeks, the water temperature in each tank was controlled, creating a temperature gradient between 14 and 25 °C. In each tank, perch larvae were the predator feeding on their zooplankton prey which fed on the phytoplankton. The perch larvae were hatched from eggs collected either in the heated area or the unheated one. Perch larvae originating from the heated area likely carried the effects of multi-generational warming. I found that they ate less zooplankton, but with a preference for larger ones compared to perch larvae originated from the unheated area. This finding suggests that the potential evolutionary effect of warming on fish can affect their prey, and likely the food webs.

In summary, this thesis demonstrates that warming induces various responses in fish, which are likely passed on to subsequent generations and can significantly impact their prey as well as other components of the ecosystem. This suggests that the effects of climate warming are unlikely to be reversible even if global temperatures cool. While it may be impossible to completely reverse the impacts of warming, advances in understanding and collaborative actions among scientists, managers, and policymakers may be the best approach to restore and protect healthy fish populations in the face of climate change challenges.

Populärvetenskaplig sammanfattning

De flesta människor har nog hört eller upplevt någon effekt av klimatförändringarna vid det här laget, vare sig det handlar om en ny rekordvarm sommardag eller allt mindre snö varje vinter. Det påverkar dock inte bara människor, utan har långtgående effekter på andra levande varelser och de ekosystem vi lever i. Fiskar är växelvarma organismer som är beroende av sin omgivning för att reglera sin kroppstemperatur och kan därför vara särskilt sårbara för att vattnet blir varmare. Många har hört talas om "survival of the fittest" — de optimistiska kan finna tröst i detta, i tron att åtminstone några "utvalda" individer kommer att överleva eftersom de lyckas "evolvera" i takt med de nya temperaturerna. Men graden av uppvärmning kan vara så extrem att den överskrider den gräns där organismer kan anpassa sig, eller att den sker så snabbt att det inte finns tillräckligt med tid för dem att anpassa sig och överleva.

För att säkerställa att fiskar överlever i sina inhemska miljöer och upprätthåller sina komplexa relationer med bytesdjur, rovdjur och konkurrenter måste vi förstå hur uppvärmning påverkar dem. Denna kunskap är avgörande för att bevara den biologiska mångfalden och förhindra att fiskar blott finns som minnen eller historiska fotografier. Vi måste fråga oss, kan fiskar evolvera som svar på stigande temperaturer? Om så är fallet, i vilken utsträckning och genom vilka mekanismer? Anpassar de sig för att bli mer värmetåliga? Kan deras avkommor ärva denna motståndskraft, vilket förbereder dem för en alltmer varm miljö? Dessutom, är det möjligt för oss att påverka eller vända denna potentiella evolutionära process?

Många forskare har studerat dessa frågor och funnit att fiskar reagerar på uppvärmning på olika sätt, såsom ökad tolerans för högre temperaturer, förhöjd ämnesomsättning, förändrat simbeteende samt förändringar i tillväxttakt och kroppsstorlek. Till exempel har vissa studier visat att fiskar växer snabbare men når en mindre storlek som vuxna efter att ha utsatts för varmare förhållanden i flera generationer. Dessa fynd tyder på en möjlig evolutionär komponent i de underliggande mekanismerna.

I denna avhandling studerade jag effekterna av långsiktig uppvärmning på fiskens evolution inom ett unikt system som består av två intilliggande, liknande kustekosystem. Ett av dessa ekosystem har värmts upp och har upprätthållit en temperatur som är i genomsnitt minst 5 °C varmare än det andra sedan 1980. Det uppvärmda området är ett 1 km² inneslutet bassäng

som tar emot utsläpp av varmvatten med en hastighet av upp till 100 m^3/s från det närbelägna Forsmark kärnkraftverk. Det inneslutna området konstruerades innan kärnreaktorerna började drivas, med avsikt att övervaka effekterna av uppvärmning på fisk och ekosystem, vilket möjliggör en jämförelse med det ouppvärmda ekosystemet i den omgivande skärgården. Denna uppställning skapar en sorts experimentuppställning med en behandling (uppvärmning) och en kontroll. Med tiden har det fungerat som en tidsmaskin, som gjort det möjligt för generationer av forskare, inklusive mig själv, att retroaktivt studera effekterna av uppvärmning på fisk och deras miljö.

Jag använde abborre, en art som är vanlig i detta system, som min modellart för denna avhandling. Abborre har kontinuerligt provtagits inom ett kontrollprogram som genomförs av Institutionen för akvatiska resurser vid Sveriges Lantbruksuniversitet (SLU Aqua) inom studiesystemet. Genom att analysera långsiktiga data på storlek, ålder och könsmognad, fann jag att abborrarna är mindre när de blir könsmogna, och deras gonader (fortplantningsorgan) är större relativt deras kroppsstorlek i det uppvärmda området jämfört med abborrarna från det ouppvärmda området. Fiskar som är mindre när de fortplantar sig för första gången tenderar att lägga färre ägg än större fiskar; emellertid kan detta kompenseras av deras mer utvecklade gonader. Jag upptäckte också att abborre kan börja fortplanta sig vid en yngre ålder, vilket kan fungera som en överlevnadsstrategi som svar på en potentiellt kortare livslängd på grund av uppvärmning. Detta mönster observerades endast i populationer som hade utsatts för uppvärmning under en eller två generationer. Abborrar som hade utsatts för uppvärmning i fem till åtta generationer, däremot, varierade mer i hur stora det var när de blev könsmogna, och blev inte nödvändigtvis könsmogna vid en yngre ålder eller hade större gonader. Denna förändring över tid reflekterar en möjlig evolutionär komponent i fiskars respons på uppvärmning.

För att utforska den genetiska grunden för fiskars evolution till följd av uppvärmning extraherade jag DNA—den genetiska informationen som kodar för de ärftliga egenskaperna och funktionerna hos organismer—från abborre i det uppvärmda och ouppvärmda området över tid. Jag valde tre tidpunkter (1980, 2000 och 2020) för att studera de senaste fyra decenniernas uppvärmning. För de första två tidpunkterna extraherade jag DNA från gällocksben från abborre, vilket är hårda, platta flikar som täcker deras gälar. Gällocken samlades in inom samma kontrollprogram som data på

abborrarnas storlek, ålder och könsmognad. Genom att jämföra den genetiska informationen mellan abborrarna från de två ekosystemen över tid identifierade jag signaler av naturlig selektion på grund av uppvärmning. Hur dessa genetiska skillnader kopplar till specifika egenskaper hos abborre, såsom deras könsmognad eller fortplantning, återstår dock att fastställa.

För att bedöma de indirekta effekterna av uppvärmning på näringskedjan genom dess påverkan på fisk, skapade jag ett miniatyrkustekosystem i 38 tankar. Genom uppvärmning höjdes vattentemperaturen i varje tank under tre veckor, vilket skapade en kontrollerad temperaturgradient mellan 14 och 25 °C. I varje tank hölls abborrlarver som åt djurplanktonbyten, vilka i sin tur levde av växtplankton. Larverna kläcktes från ägg som samlades in antingen från det uppvärmda området eller det ouppvärmda. Abborrlarver som härstammade från det uppvärmda området bar troligen spår av de många generationernas uppvärmning. Jag fann att de åt mindre mängd djurplankton, men föredrog större djurplankton jämfört med abborrlarver som härstammade från det ouppvärmda området. Detta tyder på att de potentiella evolutionära effekterna av uppvärmning på fisk kan påverka deras byten, och sannolikt även resten av deras näringskedjor.

Sammanfattningsvis visar denna avhandling att uppvärmning inducerar olika effekter hos fiskar, som sannolikt överförs till efterföljande generationer och kan påverka deras byten samt andra komponenter i ekosystemet. Detta tyder på att effekterna av klimatförändringarna på fisk sannolikt inte är reversibla ens om de globala temperaturerna skulle sjunka. Även om det kan vara omöjligt att helt vända effekterna av uppvärmning, kan framsteg i förståelsen och samarbetsåtgärder mellan forskare, förvaltare och beslutsfattare vara den bästa metoden för att återställa och skydda friska fiskpopulationer (och t.ex fortsätta att ha sill på midsommar och julbord) i ljuset av klimatförändringens utmaningar.

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Appendix

I

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ARTICLE

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Decades of warming alters maturation and reproductive investment in fish

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Abstract

How does warming affect maturation and reproductive investment in ectotherms? Younger age and smaller size at maturation, as well as altered reproduction processes, have been found in a few species subjected to elevated temperatures. These observations, however, come from studies that do not distinguish effects of warming on maturation from those on growth, are also restricted to single generation responses to warming, or have additional stressors besides warming in the study system. Here, we study warming effects on maturation and reproductive investment in wild, unexploited fish populations using a whole-ecosystem heating experiment. The experiment is conducted on Eurasian perch (*Perca fluviatilis*) in a heated and control area (with >5°C temperature difference) in the Baltic Sea. We compare female perch size at maturation using estimated probabilistic maturation reaction norms (PMRNs) and the gonado-somatic index over 17 years of heating, spanning approximately five to eight perch generations. Using the PMRN approach, we show that warming has substantial effects on maturation size independent of warming-induced changes in body growth. We found that young fish mature at a smaller size and invest more in developing their gonads in the heated population than in the unheated population. Our findings suggest that warming effects on reproductive investment may initially compensate for the cost of warming-induced decrease in maturation size caused by the trade-off between early maturation and size-dependent fecundity. After multiple additional generations of warming, maturation and reproduction traits in perch differed from those in the first generations following the onset of warming, which suggests that warming-induced evolution may have occurred. Our study is particularly relevant in the context of climate change because of the unusually large temperature difference between the areas and the fact that the heating occurred on an ecosystem level. We call for experimental studies resolving mechanisms of trait responses to warming across generations, complemented with genomic analyses, to aid understanding of organisms' long-term responses to climate change.

KEYWORDS
climate change, evolution, gonado-somatic index, life history traits, *Perca fluviatilis*, probabilistic maturation reaction norm, reproduction, size at maturation, temperature, trade-offs, whole-system experiment

INTRODUCTION

Temperature has a pervasive influence on ectotherms, whose body temperature depends on their ambient thermal environment. Temperature directly influences physiological traits such as metabolic rate, growth rate, development rate, and hormone regulation, and key processes such as survival (reviewed in Angilletta, 2004; Huss et al., 2019). Still, temperature effects on key life history traits such as age- and size at maturation and energy allocation to reproductive versus somatic growth are not well understood (Audzijonyte et al., 2016). Age- and size at maturation are to a large extent determined by juvenile growth rate and in turn, affect adult growth rate, lifetime reproductive success, and mortality (Berrigan & Charnov, 1994; Stearns, 1992). As resources are limited, the allocation of energy to reproduction upon maturation will lead to slower somatic growth, especially in organisms with indeterminate growth, such as fish. Hence, fish adult size is largely dependent on size at maturation. Larger size at maturation in fish is often linked to higher fecundity, higher offspring fitness, and lower mortality due to lower predation risk following the larger body size. However, a larger (adult) body size also costs fish more energy and resources to maintain (Roff, 1992). The opposite holds for smaller size at maturation, which is associated with smaller adult body size and lower fecundity (Roff, 1992), but also lower maintenance costs. Moreover, the differences in maintenance costs between large- and small-sized fish increase if the temperature is high (Lindmark, Ohlberger, et al., 2022). Early maturation, commonly concurring with smaller maturation size, can increase the number of reproduction events in a lifetime for multiple spawning fish. Smaller maturation size is thus the result of optimizing fitness by balancing fecundity and predation risk against maintenance cost and potentially, reproductive lifespan. Reproductive investment, that is, energy allocated to reproduction such as developing gonads, is another trait that directly affects fish reproduction and recruitment success (Rosecchi et al., 2001). Such investment is traded off against somatic growth, survival, and future spawning success (Kozlowski, 1996; Stearns, 1992). Because the processes underlying these trade-offs depend on temperature, warming could induce changes in these key life history traits. Due to global warming's potentially profound impacts on populations and food webs (Audzijonyte et al., 2013), it is especially important to understand how warming affects fish maturation and reproductive traits, including both immediate and long-term responses.

Maturation describes the process of an organism reaching maturity, whereas maturity is the life stage an organism enters thereafter. Fish often need to exceed a size threshold to mature (Hutchings, 2002; Figure 1a,d). Warming could therefore cause fish to mature earlier (at a younger age) if growth rates increase with temperature (Angilletta, 2004; Berrigan & Charnov, 1994; Sandström et al., 1995; Figure 1d). If the size threshold for maturation also depends on age, changes in growth would alter both age- and size at maturation (Figure 1e,f). Growth rate related changes in age- and size at maturation induced by warming have been supported by theoretical models (Zuo et al., 2012) and observed in controlled experiments (Jonsson et al., 2013). In the wild, warming has been associated with both decreased age- and smaller size at maturity in fish (Ottersen et al., 2006; Shapiro Goldberg et al., 2019). The opposite, increased water temperature leading to later fish maturation, has however also been observed (Otero et al., 2012), including larger size at maturation after multiple generations of experimental warming (Loisel et al., 2019). Changes in maturation traits can thus occur as a direct consequence of warming effects on growth.

Evidence shows, however, that temperature can affect fish maturation independently from its influence on juvenile body growth (Dhillon & Fox, 2004; Kuparinen et al., 2011). By quantifying the probability of an individual to mature using probabilistic maturation reaction norms (PMRNs; Dieckmann & Heino, 2007), such direct impacts of warming on maturation can be disentangled from changes related to juvenile growth rates or mortality. Direct effects of warming on maturation can arise through responses in physiological processes, shifting the PMRNs without altering body growth (Figure 1a–c). Such responses are likely associated with higher temperature, which modifies fish endocrine profiles and affects gonad development (Kraak & Pankhurst, 1997) and increased development rates (Wootton et al., 2021). If temperature increases further, however, the reverse response—larger size and older age at maturation—can be observed (Dhillon & Fox, 2004), likely because temperatures exceed species' maximum tolerance. Warming can thus affect maturation size and age via physiological changes

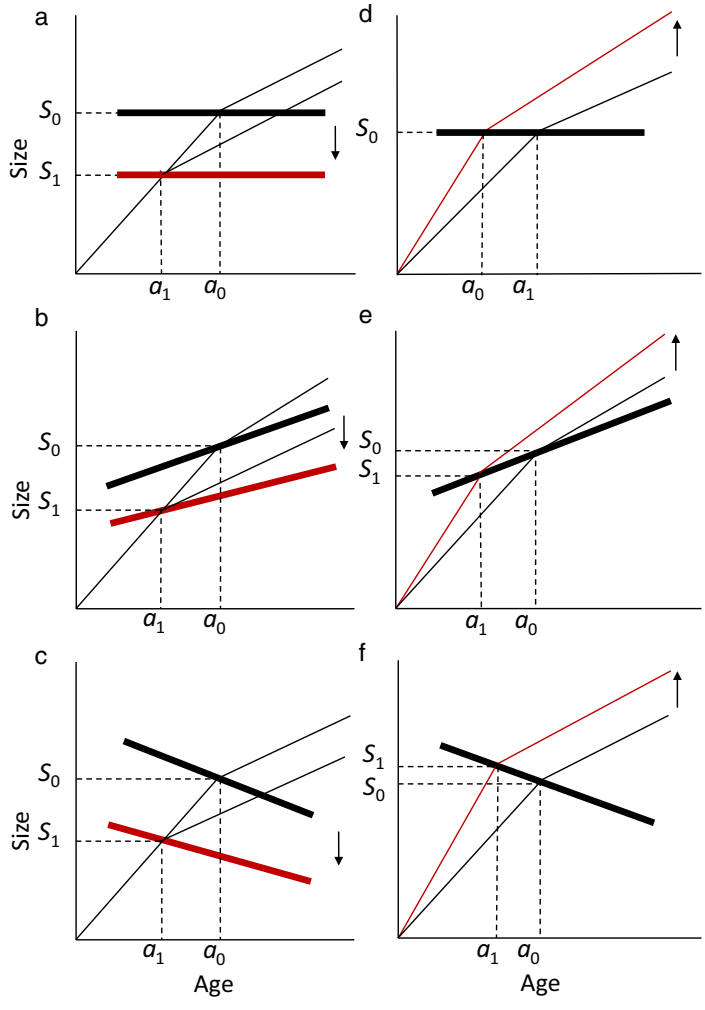

FIGURE 1 Probabilistic maturation reaction norms (PMRNs; thick lines) reflect direct changes in maturation schedules of individuals in relation to their age and size, independent of changes in juvenile growth (thin lines) rates or mortality. Changes (from black to red lines; follow the directions of the arrows) in both PMRNs (thick lines in subplots a, b, and c) and growth rates (thin lines in d, e, and f) can alter age and/or size at maturation (from a_0 to a_1 and/or s_0 to s_1), whereas changes in growth rate will not affect the PMRNs (d, e, and f).

in growth or maturation reaction norm, or both (Figure 1).

Can warming also induce evolutionary changes in phenotypic maturation traits in addition to them changing plastically? Size- and age at maturation have genetic components in fish (Hutchings, 2002). As warming can induce strong phenotypic responses in fish age- and size at maturation (Dhillon & Fox, 2004; Kuparinen et al., 2011; Loisel et al., 2019), it is likely a strong selection pressure and thus a potential evolutionary driver (Crozier & Hutchings, 2014). Most experiments studying warming effects on fish maturation, however, focus on single generation responses. To our knowledge, no study has analyzed the effect of warming lasting multiple generations on maturation traits, independent of effects via growth, in wild fish populations.

In this study, we analyze how warming affects fish maturation and reproductive investment in Eurasian

perch (*Perca fluviatilis*) over 17 years (approximately five to eight generations) in an unexploited wild population that has been consistently subjected to elevated water temperatures. Perch sampled from an artificially heated, enclosed coastal area were compared with those from an adjacent control area with natural water temperatures. We examined whether and to what extent multigenerational warming has (1) caused fish to mature at a smaller size or younger age, independent of growth effects (by using PMRNs); (2) altered reproductive investment as indicated by the mass of their gonads relative to their body weight; and (3) if the effect of warming on these traits has changed over time.

MATERIALS AND METHODS

Study area and species

We studied effects of warming on fish maturation and reproduction using a whole-ecosystem experimental setup with a chronically and artificially heated enclosed coastal area and its adjacent unheated control area in the Baltic Sea (Appendix S1: Figure S1). The enclosure was constructed in 1977 with the intention to study the future effect of heated water discharge from a nuclear power plant on fish and other organisms in comparison with an unheated area used as control (Thoresson, 1992). Since 1980, the enclosure has been receiving cooling water discharge (flow rate of 80–100 m^3/s), making its water temperature 5–10°C higher than in the adjacent control area (Appendix S1: Figure S1). A grating at the outlet of the heated area prevented the exchange of fish bigger than 10 cm between the areas (Adill et al., 2013). Its removal in 2004 increased the probability of larger organisms dispersing between the areas, although the strong current likely prevents the immigration of small or poorly swimming organisms into the heated area. Any kind of exploitation, except for test fishing (see below), has been forbidden in both areas since 1977. The most abundant fish species in both areas is Eurasian perch (Adill et al., 2013). Female perch can become mature from two to five years of age (Heibo & Magnhagen, 2005; Sandström et al., 1995). The onset of spawning in perch depends on temperature and in our study area usually takes place in March to early June (Lukšienė et al., 2000), following gonad development from late autumn to spring (Scharnweber & Gårdmark, 2020). There are considerable regional genetic differences between Baltic Sea perch populations, which can be related to perch being a stationary species with natal homing behavior, variation in temperature, and other environmental variables (Olsson et al., 2011). Perch populations in the heated and control areas show both phenotypic and genetic differentiation. In the heated area, perch have larger size with age, higher growth rates when small (Huss et al., 2019), higher mortality (Lindmark, Karlsson, et al., 2022; Lindmark, Ohlberger, et al., 2022), and more advanced gonad development at a given time of year (Lukšienė et al., 2000). Within the first five years of heating, the youngest age and smallest size at maturity (which is different from *maturation*) have declined for perch in the heated area in comparison with perch in the unheated area (Sandström et al., 1995). Following the separation from the original population, the perch population in the enclosed area has shifted the allelic composition of MHC class II genes related to selection imposed by parasites (Björklund et al., 2015) and has higher expression levels of mitochondrial genes than perch in the surrounding area (Pichaud et al., 2020). Perch in the heated area has thus diverged phenotypically, and possibly also genetically, from perch in the adjacent unheated control area.

Data

Test fishing with a consistent type of multi-mesh gillnets has been conducted regularly to monitor life history traits of perch in the unheated area since 1970 and in the heated area since 1977 (i.e., after its construction). Test fishing is carried out in parallel in both areas at the same depth range and similar distances to shore (Thoresson, 1992). The secchi depth, often used as a eutrophication indicator, is at similar levels in both areas (Sandström & Karås, 2002 and more details in Appendix S1: Comparison of the areas: similarities). Thus, not only are the two areas adjacent to each other and share the same air temperature, but they are generally also subjected to similar levels and trends in key Baltic Sea environmental drivers (HELCOM, 2009, 2013).

To compare short- and long-term heating effects within this time series, we chose to focus on female perch of age two to five born during the two time periods 1980–1984 and 1991–1996 to estimate their maturation schedule and reproductive investment. We chose these periods to be as distant in time as possible while still being before the grating removal in 2004, so that perch in the two areas were still physically separated. The periods and age range of the perch chosen jointly guaranteed that all perch were caught no later than 2003, such that the exchange and gene flow between the heated and unheated areas perch was minimal. This resulted in that the two periods are 7 years apart, equivalent to a separation by about two to three generations of perch (based on

perch age at *maturity* in this area, Sandström et al., 1995, because there is no information on age at *maturation*), allowing us to study warming-induced changes in maturation and reproductive investment occurring between multiple generations. We group perch born in different years into two periods to enable large enough sample sizes as well as the presence of both immature and mature perch at each age in each area during each period, which is required for robust calculations of PMRNs (see *Probabilistic maturation reaction norms*, below). In the first period, 1980–1984, in the heated area, perch were either the first generation to be exposed to heating or offspring of those who had been exposed to heating for about one or two generations. In the later period, 1991–1996, they were all offspring of perch that had been exposed to heating since 1980, that is, for 11–17 years, which is equivalent to about five to eight generations. In total, 3060 perch were sampled, and there were more than 400 individuals sampled per heated or unheated area and per first or second period (Appendix S1: Table S1). We focus on females because only their sample size enabled analyses of heating effects over multiple generations, whereas there are none or too few ages of immature males from the period during which they were sampled (Appendix S1: Table S1). As perch displays sexual size dimorphism (Heibo & Magnhagen, 2005), our sex-specific analyses also ensure that any changes in maturation and reproduction are not confounded by shifts in sex ratio in the population or samples.

Measurements of sampled females were carried out identically in both areas, including body size at capture (in millimeters), age (discrete year), and back-calculated size at each age (in millimeters) from measurements of growth ring distances on the operculum bone, gonad weight (to the nearest 0.01 g), maturation status (mature/immature) from gonad examination, and total body weight (in grams) (see Appendix S1 for detailed descriptions of calculations and examinations).

Reproductive investment is commonly indicated by gonad mass relative to total body weight, the so-called gonado-somatic index (GSI = gonadal weight/total body weight × 100%; Rizzo & Bazzoli, 2020). Female perch GSI from the selected cohorts ranged from 0% to 30.6% (Appendix S1: Figure S2). To capture potential changes in gonad investment among spawning individuals, we filtered out perch sampled well outside the spawning season because their gonads would likely be nondeveloping or spent. We therefore chose sampling weeks 10–30 to capture the peak of gonad development (Appendix S1: Figure S2). To separate prespawning gonads from undeveloped ones, we examined GSI distribution during these weeks. It is bimodal (see Appendix S1: Figure S3) with the two clusters separated at GSI ≈ 10%. We therefore assigned perch with GSI > 10% as mature with prespawning or spawning stage gonads for the analyses.

Maturity ogives

Maturity ogive ($o(a, s)$), the proportion of mature individuals in a population in each age group (a) and size class (s), is commonly estimated in fish stocks to provide information about reproduction and fecundity per age group. Because of the binary nature of maturation status (being immature or mature), maturity ogive is often modeled as a probability using the logistic regression as a function of age, or size, or both. Once the relationship between maturity ogive, age, and size is estimated in a population, the probability of an individual of any age and size in the population *being* mature can be predicted (Heino et al., 2002). As we need to estimate the PMRNs (see the below section) of individual perch from the two areas and two periods, maturity ogive of an individual at age a (which is the age at capture) and age $a-1$ are needed (Heino et al., 2002). To best describe maturity ogive as a function of age and/or size for each area and period separately, we employed model selection using the logistic regression model (Equation 1) and data on maturation status (0 or 1), age (a), and size (s) at capture of perch sampled from each area and period,

$$\text{logit}[o(a,s)] \sim \alpha_0 + \alpha_1 a + \alpha_2 s + \alpha_3 a \times s, \quad (1)$$

where α_1, α_2, and α_3 are estimated parameters. We selected the best model for each dataset from models including all combinations of age, size, and their interaction as the one with the lowest Akaike information criterion (AIC; Burnham & Anderson, 2002; Appendix S1: Table S2). To allow for comparisons between areas and periods, we used $o \sim \alpha_0 + \alpha_1 a + \alpha_2 s + \alpha_3 a \times s$ as the best model throughout to predict maturity ogives for the PMRN calculations in the next step (Appendix S1: Figure S4). Model assumptions were checked using diagnostic plots (Appendix S1: Figures S6 and S7). We estimated Nagelkerke's R^2 for each model (Appendix S1: Table S2) as a measure of the variation explained (Nagelkerke, 1991).

Probabilistic maturation reaction norms

Data on perch body size and maturity status at capture give no direct information on the maturation schedule of an individual, that is, the exact age and size when fish

have or will become mature. It is important to appreciate the distinction between age- and size at *maturation* and age- and size at *maturity*. The latter can be the age and size of any already mature individual, information that can be easily obtained at capture. We, however, lack data on the former because gonad examination at capture can only provide information on whether a perch has become mature or not but reveals no information about whether a mature fish became mature last year or many years ago. However, estimating PMRNs—the probability of an individual maturing at a given age as a function of size—enables us to investigate individual maturation schedules, for example, size at maturation per age, and by comparing the heated and unheated population, how warming has affected them independently from changes in maturity resulting from changes in growth or mortality. We derived PMRNs for each individual using its size at capture, age, and growth (back-calculated size at age) and the maturity ogive model (Equation 1) to predict its corresponding maturity status. We then calculated the probabilities of an individual becoming mature $m(a, s)$ at a given age a and size s using

$$m(a,s) = \frac{o(a, s) - o(a-1, s-\Delta s(a))}{1 - o(a-1, s-\Delta s(a))}, \quad (2)$$

where $o(a, s)$ and $o(a-1, s-\Delta s(a))$ are the maturity ogives of fish at a given age and size in the year of capture and the previous year, respectively, and $\Delta s(a)$ is the individual growth increment in between (Barot et al., 2004). We focus on two- to five-year-old females, as perch are commonly found to be mature at these ages, and this age range meets the data requirements for the Barot et al. (2004) approach best. Most importantly, this approach requires the presence of both immature and mature individuals at each age, and at each age, there should be at least 100 individuals altogether. Instead of fitting von Bertalanffy's growth curves to calculate an average size-at-age of the population, we used individual growth data, that is, back-calculated individual size-at-age a and $a-1$ derived from their size at capture and operculum structure (for details see Appendix S1). Using these individual growth trajectories rather than a population mean size at age, we calculate each individual's probability of maturing, $m(a, s)$, rather than a population mean $m(a, s)$ per age, which is a population mean PMRN. This reveals variation in PMRNs among individuals between areas and periods, thereby providing more information on changes in maturation schedules than the commonly available population mean in studies of PMRNs (e.g., Vainikka et al., 2009). Therefore, we are able to study how warming may affect not only mean but also within-population variation in perch maturation.

Statistical modeling of PMRNs and GSI

To investigate if warming and duration of warming affect probability of maturing (and hence size at maturation), we modeled $m(a,s)$ as a function of area (heated or unheated), time period (early, 1980–1984, 5-year warming or late, 1991–1996, after multigeneration warming), age (2–5), and size using binomial generalized linear models (GLMs). Model assumptions were checked, and validation was performed using diagnostic plotting (Appendix S1: Figure S8). Pairwise differences in maturation probabilities between the heated and unheated areas and time periods were assessed using nonparametric Mann–Whitney U test. The early period would mainly represent plastic effects of warming as most perch were the first or second generation to experience warming. In the late period, most perch were about the fifth or sixth generation that had been subjected to warming, which would therefore reflect long-term and potentially evolutionary warming effects.

We fitted GSI as a function of area, time period, age (3–5), size, and all combinations of the interactions of the four terms using GLMs. Due to scarcer sampling of gonad weights than gonad status, less GSI data was available for two-year-old perch (Appendix S1: Table S3). We assumed a Gaussian-distributed residual pattern for the models as the data captured the gradual gonadal development from autumn to spring. Student t-tests were used to assess differences in GSI between the areas or periods. Model validation was checked by inspecting diagnostic plots (Appendix S1: Figure S9).

For both $m(a,s)$ and GSI, we ran null and full models, respectively, as a function of only age, size, and age × size and as a function of age, size, area, period, and all their possible interactions. We selected the models manually through a stepwise process, working backward from the full model (Appendix S1). We consider the best model to be the one with the lowest AIC, and all models with $\Delta\text{AIC} < 2$ to that model to be indistinguishable. To illustrate consequences of maturation probabilities for maturation size, we derived the predicted perch body size with 50% probability of maturing (Lp50) for each age, using the best model. All data processing and statistical analyses were conducted in R, version 4.0.2 (R Core Team, 2014). Data visualization and processing were done using packages within the tidyverse collection (Wickham et al., 2019). Derivation of Lp50 from the best PMRN model was done using package "rje" (Evans & Drton, 2022).

Whether area or period is included in the best model for either maturation probabilities or reproductive investment indicates whether warming or its duration plays a role for fish maturation and reproduction.

RESULTS

Maturation

Area (heated vs. unheated) was retained in the best PMRN model (Table 1, Appendix S1: Table S4; as well as in all models with ΔAIC < 2, Appendix S1: Table S8), implying that temperature has affected the maturation probability of female perch (Figure 2). The heating effect on maturation has also changed over about five generations, as the duration of heating (period) was included in the best model (Table 1). Both area and period were included in the best model as well as 2-, 3-, and 4-way interactions with age and size. Importantly, this suggests that heating effects varied over ontogeny and across generations.

Both the probability of becoming mature (Figure 2) and the predicted maturation size (Lp50; Figure 3), differ between the heated and unheated areas. Within the first five years of warming, fish of a given body size have a higher probability of maturing at early ages (Figure 2a,b; Mann–Whitney U test, $p < 0.05$, $\eta^2_{age2} = 0.30$, $\eta^2_{age3} = 0.08$) and thus mature at a smaller size at age two and three in comparison with fish in the control area (Figure 3a). Over age, the difference in $m(a, s)$ between perch in the areas decreases, such that predicted Lp50 at age four and five in the two areas are more similar (overlapping CI in Figure 3a; see Appendix S1: Table S5 for significance level and effect size).

Compared with the Lp50 of perch born during the first five years of warming, perch Lp50 is even smaller after multigenerational heating at age two to four (Appendix S1: Figure S10; $p < 0.05$, η^2 is 0.30, 0.39, and 0.04 for respective ages). Two-year-old perch in the heated area showed an even greater probability of maturation ($p < 0.05$, $\eta^2 = 0.73$; Figure 2e), and thus a smaller maturation size (Figure 3b), than same-aged contemporaries in the unheated area. In contrast, for three-year-old perch the maturation size was similar in both areas (overlapping CI at age three; Figure 3b). Interestingly, four- and five-year-old perch in the heated area had lower probability of maturing ($p < 0.05$, η^2 is 0.70 and 0.64 for age four and five; Figure 2g,h) and larger maturation size (Figure 3b) than those in the unheated area. Moreover, after the multigenerational heating, the variation in probability of maturing for three- to five-year-old perch is substantially greater than for perch in both the unheated area and those that have only experienced the initial five-year heating (Appendix S1: Figure S11). Notably, maturation size changed between the two periods in the unheated area as well. That is, in the late period, perch matured at smaller size at all ages compared with the early period (Figure 3).

Reproductive investment

Area was retained in the best GSI model (Table 2, Appendix S1: Table S6, as well as in all models with ΔAIC < 2 to the best model, Appendix S1: Table S9), implying that temperature is important to explain female perch reproductive investment. Different levels of interaction between area, period, age, and size were also included in the best model (Table 2). Perch in the heated area invested relatively more in reproduction at age four after five years of heating than perch in the control area ($p < 0.05$, Cohen's $d > 1$; Figure 4a). GSI of five-year-old individuals, however, overlapped substantially between the

TABLE 1 Model selection results for probability maturation reaction norms of two- to five-year-old female perch across both areas (heated vs. unheated) and periods (early 1980–1984 and late 1991–1996).

Model	Formula	df	AIC	Residual deviance[a]
Null	m ~ age + size + age:size	2951	3301	619
Full	m ~ age + size + age:size + area + age:area + size:area + age:size:area + period + area:period + age:period + size:period + age:size:period + age:area:period + size:area:period + age:size:period:area	2939	2152	150
Best	m ~ age + size + age:size + area + age:size:area + period + area:period + age:period + age:size: period + age:area:period + size:area:period + age: size:period:area	2942	2143	153

Note: Null model (without effects of area or period), full model (effects of age, size, area, and period and all their interactions), and the best model based on the lowest AIC are displayed (for models with AIC within 2 units of the best model's; Appendix S1).
Abbreviation: AIC, Akaike information criterion.
[a]The null deviance of the model that includes only the intercept is 863.

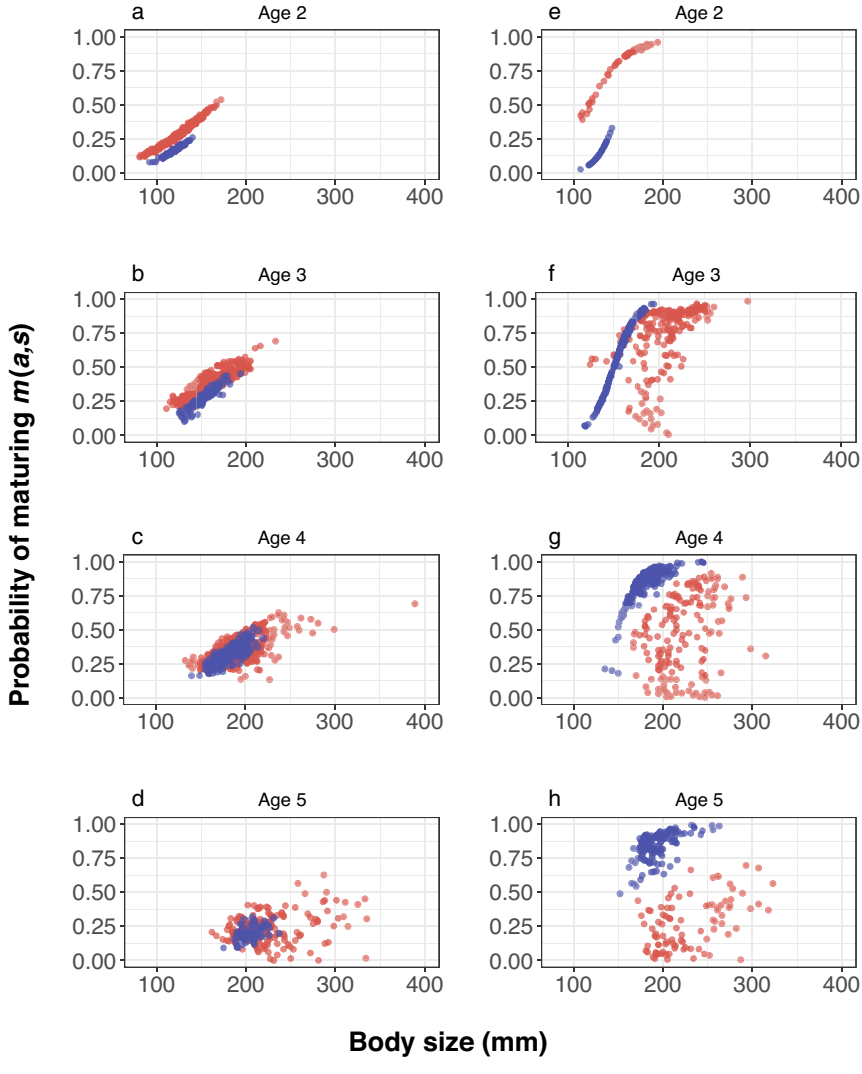

FIGURE 2 Probabilities of maturing ($m(a,s)$) of female perch from the heated coastal area (red) and the adjacent unheated area (blue) for two-year-old (subplots a and e), three-year-old (b and f), four-year-old (c and g) and five-year-old (d and h) individuals. Maturation probabilities differ between perch from the early period (a–d) following the onset of heating (cohorts 1980–1984) and the late period (e–h), after about five to eight generations of heating (cohorts 1991–1996).

areas ($p = 0.57$; Figure 4b). The effect of warming on GSI changed over time (Appendix S1: Table S7). After multigenerational heating, GSI was similar in the two areas for both four- and five-year-olds ($p > 0.5$; Figure 4c,d). In the heated area, four-year-old perch had smaller GSI after the long-term warming than after only five years of warming ($p < 0.01$, Cohen's $d = 0.86$; see Appendix S1: Figure S12).

DISCUSSION

We found that warming has a direct impact on both fish maturation and reproductive investment in the wild, beyond shifts caused by temperature-induced changes in growth. Fish in the heated area generally had a higher probability of maturing, hence a smaller size at

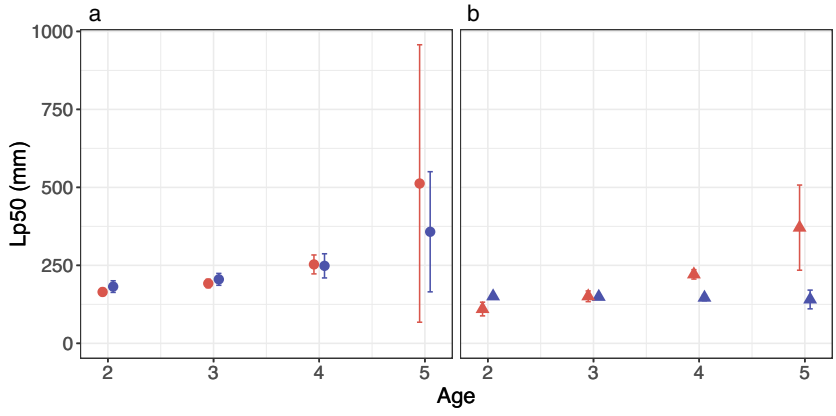

FIGURE 3 Body size (mm) of female perch with 50% probability of maturing (Lp50), for two- to five-year-olds in the heated (red) and unheated area (blue), for the early period (subplot a, cohorts 1980–1984; circles) and the late period (b, cohorts 1991–1996; triangles). The 95% confidence interval of Lp50 for each age, area, and period combinations are illustrated as bars.

TABLE 2 Model selection results for selection of variables explaining the building up of gonads of three- to five-year-old female perch, across both areas (heated vs. unheated) and time periods (early 1980–1984 and late 1991–1996).

Model	Formula	df	AIC	Residual deviance[a]
Null	GSI ~ age + size + age × size	446	2383	5134
Full	GSI ~ area × age × size × period	434	2362	4652
Best	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:area + age:size:period + age:area:period + size:area:period	437	2358	4672

Note: The null, full, and best models are displayed here (models with AIC within 2 units of the best model can be found in Appendix S1).
Abbreviation: AIC, Akaike information criterion.
[a]Null deviance for the model including only intercept is 5183.

maturation, and initially also invested more in reproduction than fish in the unheated population. The temperature effect on maturation size and reproductive investment varied over fish ontogeny, as shown by different responses in these life history traits to warming in fish of different ages. By tracking trait changes in perch across generations under constant heating, we also found that the effect of warming on both traits varied over time, and had a different relationship with age (ontogeny) after multiple generations of heating. This suggests that warming-induced evolution may have occurred, but evidence on underlying adaptive genetic changes is needed.

Within the initial five years of heating, young female perch in the heated area were more likely to mature at a smaller size and have larger relative gonad size than those in the unheated population. Warming-induced decrease in size at maturation, beyond any effect of growth, has been found in several fish species under laboratory conditions when studied within a single generation (Dhillon & Fox, 2004; Kuparinen et al., 2011). Uniquely to our study, we also found this in a wild fish population exhibiting multigenerational heating at an ecosystem scale. Warming may directly alter maturation-regulating hormones and development rates at different life stages (Miranda et al., 2013; Wootton et al., 2021), resulting in advanced maturation. Higher food availability, therefore improved body condition, is also associated with higher size-specific maturation probabilities, independent of growth (Uusi-Heikkilä et al., 2011). In our system, perch food abundance in the heated area may have increased initially due to warming (Sandström, 1991; whereafter we lack prey data), although this may not always be the case in other heated systems. Thus, the higher probability of maturation and smaller maturation size could result from both a direct effect of higher temperatures on fish physiology and an indirect effect via improved food conditions. Given more food in the heated area, perch might also be better able to afford diverting of

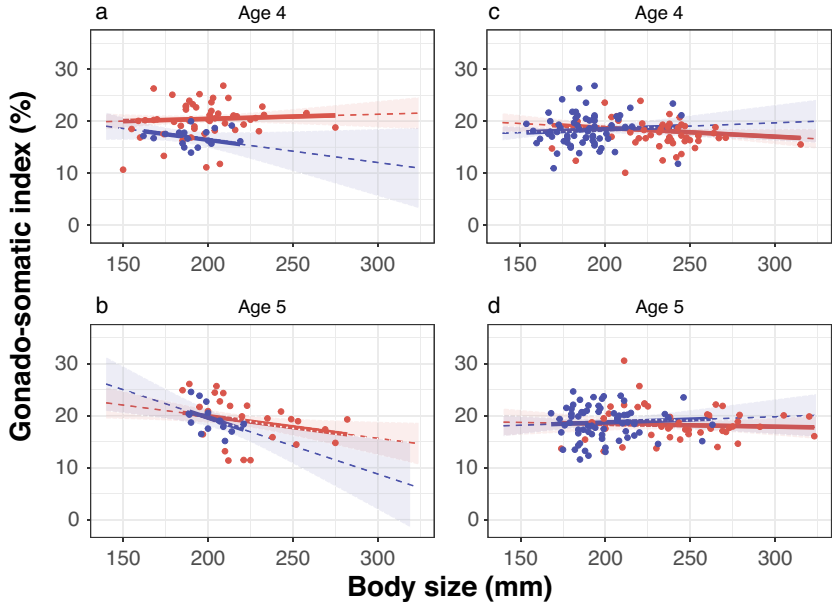

FIGURE 4 Gonado-somatic index (GSI) predicted for female perch from the heated area (red; solid part of the lines corresponds to the size range of the empirical dataset) and from the unheated area (blue) for age four (a and c) and five (b and d), respectively, for the early period: cohorts 1980–1984 (a and b) and the late period: cohorts 1991–1996 (c and d). The 95% confidence intervals are added for the whole length range as shading. Observed GSI of perch are shown as red (heated area) and blue (unheated area) points.

energy to reproductive investment (McBride et al., 2015) at a smaller size, explaining the increased GSI (Wootton et al., 2022). Previous studies found a negative relationship between increased temperature and absolute gonad size in ectotherms (Donelson et al., 2010), but temperature effects on relative gonad mass (as in our study) are rarely looked at. Larger relative gonad size can be beneficial in warmer environments if the increase in gonad size corresponds to increased egg size, which can result in higher offspring survival in warmer waters (Jonsson & Jonsson, 2019). Another explanation is that investing more energy into reproduction at a young age might compensate for lowered fecundity due to the smaller body size that results from the warming-induced decrease in size at maturation and thus smaller adult size (Roff, 1992). This largely corresponds with our findings in the first five years of warming. Although for the youngest ages displaying the greatest reduction in maturation size, our sample size for gonad weights was too small to allow comparisons between areas. Our whole-ecosystem warming experiment reveals that warming for up to two generations can decrease maturation size. To our knowledge, few studies have addressed long-term warming impacts on both maturation schedules and reproductive investments (but see Wootton et al., 2022 for a laboratory experiment on this). Our joint analyses of these suggest that reproductive investment of fish in the wild may respond to warming in relation to concurrent changes in size at maturation.

In the heated area, after long-term (from 1980 to 1991–1996) warming, corresponding to at least three additional generations of perch exposed to warming following the first five years of warming, maturation size of perch of all ages decreased compared with perch born during the initial years of warming (from 1980 to 1984). This is the first time that warming has been associated with changes in fish maturation over generations in the wild, independent of growth effects induced by warming. Shifts in the PMRNs, following multigenerational warming, could result from a heritable genetic component associated with selection favoring a decrease in maturation size, as inferred in PMRN studies on other selection pressures (reviewed in Dieckmann & Heino, 2007; Gobin et al., 2021). From a life history perspective, smaller maturation size can be beneficial under warming, as it results in a smaller adult size when energy is diverted to reproductive development than somatic growth (Roff, 1992). The benefits can be lower maintenance costs (Forster

et al., 2012), especially in warming waters where the metabolic rate of ectotherms inevitably increases. A younger age at maturation can enable more reproductive events, which can increase reproductive success in unpredictable environments (i.e., bet hedging; Slatkin, 1974). Furthermore, if lifespan decreases due to a warming-induced increase in mortality (Pershing et al., 2015), it may be beneficial to mature and spawn as early as possible. The increased mortality in our heated area (Lindmark, Karlsson, et al., 2022; Lindmark, Ohlberger, et al., 2022; Sandström et al., 1995) may thus partly explain the smaller perch maturation size therein. We cannot, however, rule out maternal effects (e.g., silver spoon effect; reviewed in Jonsson & Jonsson, 2014). Genome-wide screens of footprints of selection of perch from the two areas sampled would be one potential step to distinguish if warming has exerted selection on genetic components.

In contrast to most PMRN studies, we derived maturation probabilities for individual fish using back-calculated individual size at age trajectories instead of predicted mean sizes at age based on a growth model fitted at the population level. This enabled us to reveal that warming has affected variation in maturation within the heated population. After multigenerational heating, variation in maturation probability increased for three- to five-year-old perch. This would be expected if fish have adapted to long-term warming using alternative evolutionarily stable strategies (some maturing at smaller size with higher fecundity, others maturing at larger size but with a smaller reproductive investment). This supports findings from a laboratory experiment showing that fish had larger size at maturation and higher fecundity after six generations of warming than fish that experienced warming for a single generation (Loisel et al., 2019). Alternatively, the variation in maturation can stem from the fact that water temperature in the heated area exceeded perch optimal temperatures (especially for large-sized individuals; see fig. S1 in Huss et al., 2019) in some years (e.g., >30°C; Appendix S1: Figure S1). Given that excessively high temperatures can postpone maturation (Dhillon & Fox, 2004; Miranda et al., 2013), a variable frequency of extreme temperatures between years can cause increased size at maturation for first-time spawners in some cohorts but not others. Ideally, experiments with controlled temperature treatments at different locations along the thermal curve, pursued over generations, should be carried out to test this. The observed warming-induced increase in maturation variation demonstrates the importance of applying back-calculated individual growth trajectories in PMRN analyses in general and of addressing warming impacts on not only means but also on variation within populations.

Interestingly, the decreased maturation size of perch in the heated area compared with that in the unheated area weakened at older ages within the five years of heating, and changed direction after multigenerational warming. This could result from the increased variation in maturation probability in the heated area. It might also be partly due to a decrease in perch maturation size at age four and five over time in the unheated area. Although the unheated area was chosen and paired with the heated area as a "control" for the increased temperature "treatment", it is open to the surrounding sea. Therefore, perch may have been subjected to a range of different selection pressures, such as exposure to natural predators with different predation pressures, on which we lack data to compare between the areas. It is unlikely that responses in the control area are due to temperature increase induced by water exchange with the adjacent heated area, as the design of the enclosed bay ensures this exchange is small (Sandström et al., 1995; Appendix S1: Figure S1). Both areas have been subjected to climate change, however, the temperature increase between the two study periods is small (<1°C/decade in the Baltic region; HELCOM, 2013) and much smaller than the temperature difference imposed by the artificial heating (Appendix S1: Figure S1). We cannot offer a definite explanation for the changes observed in the unheated area, as our heating experiment at the ecosystem scale suffers from the fact that there is only one heated ecosystem and one control ecosystem. Factors other than water temperature may have contributed to the differences between the control and heated areas. However, for factors for which data were available and based on the same type of measurements in both areas, that is, perch population density (as indicated by catch per unit effort), fish community composition, and water quality (as indicated by secchi depth), the differences between the areas do not correlate with warming (see Appendix S1: Comparison of the areas: similarities). Furthermore, perch sampling took place at the same water depths and distances to shore in the heated and unheated areas (Appendix S1: Figure S1), both free of fishing pressure. More importantly, the temperature difference (>5°C) between the treatment area and the control area is uniquely large and persistent over multiple generations for a natural system, especially if compared with the less than 1°C temperature increase induced by climate change in the Baltic region over the study period (HELCOM, 2013). These all suggest that the difference in temperature is the key difference between the areas and that changes observed between the artificially heated and unheated areas and in the heated area over multiple generations were likely caused by the substantial (+5–10°C; Appendix S1: Figure S1) warming.

We have to interpret our results somewhat cautiously due to a few limitations in sampling. Our sample of the youngest and oldest ages in both areas contains somewhat fewer fish than what would be ideal according

to the method used (Barot et al., 2004; Appendix S1: Table S1). This might have caused the low variance explained of the ogive models (Appendix S1: Table S2). Alternatively, the reason of "low fit" ogive models could be that we were unable to incorporate body condition (which impacts fish maturation ogives; Uusi-Heikkilä et al., 2011) in our models due to a lack of body weight data. Because warming has increased perch body growth in the heated area over time (Huss et al., 2019), they were larger than perch from the unheated area in the late period but not in the early period (Appendix S1: Figure S13). This can explain the GSI clustering (that there were no small perch with developing gonads in the heated area and no big perch with developing gonads in the control area), which may have limited our ability to find differences in GSI between the areas. We selected identical sampling weeks ("prespawning period") for both areas for the GSI analysis. Warming can however shift spawning phenology in fish (Miranda et al., 2013) and such shifts have been observed in our case (Appendix S1: Figure S2; Lukšienė et al., 2000), which means more perch were sampled closer to spawning in the heated area than in the unheated area. This may explain the larger GSI found in the heated area. However, due to the different sampling schedules in the areas, it is difficult to compare the temporal dynamic of gonad development over the entire reproductive cycle between the areas. While these aspects limit our ability to infer the actual mechanism of increased GSI due to warming, our main finding that warming increases the probability of maturation and reduces maturation size in young fish holds.

Increased maturation probability and smaller maturation size due to warming are likely to affect both ecological and evolutionary dynamics. A younger maturation age implies shortened generation time, which can accelerate the speed at which evolutionary processes occur (Roff, 1992). If the cause of the smaller maturation size is evolutionary, the directional selection caused by warming can make the population lose genetic variation. As the elevated temperature regime in the heated area corresponds to the projected increase of 2–4°C in the Baltic Sea surface temperature until the end of the century (HELCOM, 2013), our findings based on a whole-ecosystem heating experiment make the case for that climate change will impact fish maturation size. A warming-induced decrease in maturation sizes results in smaller mean adult sizes in the population, which potentially decreases recruitment capacity (Hutchings, 2002) and population biomass production (van Dorst et al., 2019). Decreased sizes can also cause changes in predator–prey interactions, affecting the overall food web and ecosystem functioning (Lindmark et al., 2019).

In conclusion, our study demonstrates multigenerational warming effects on maturation and reproductive investment on an unexploited, wild fish population based on a large sample size. We found a strong increase in maturation probability, that is, a decrease in maturation size, in response to warming over a five-year period. Interestingly, this decrease intensified after multiple generations of warming, suggesting evolutionary change. Parallel to a decrease in maturation size, we found that reproductive investment increased after five years of warming, however, this difference disappeared after multiple generations of warming. Our results emphasize that warming impacts on organism maturation can vary both ontogenetically and over time, involving potentially both phenotypic and genotypic responses, and also be linked to other life history traits. We call for future experimental studies looking into effects of increased temperature on ectotherm growth-independent maturation schedules over multiple generations, coupled with investigations of corresponding genomic changes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data and code (Niu et al., 2022) are available from Zenodo: https://doi.org/10.5281/zenodo.7351524.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary materials

Decades of warming alters maturation and reproductive investment in fish

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Contents

Study area: ecosystem warming experiment

The whole-system heating experiment consists of a heated treatment: an enclosure ("Heated area" in Figure S1) receiving cooling water from two reactors of the nuclear power plant Forsmark, and a control: an adjacent area ("Unheated area" in Figure S1) with natural Baltic Sea water temperature.

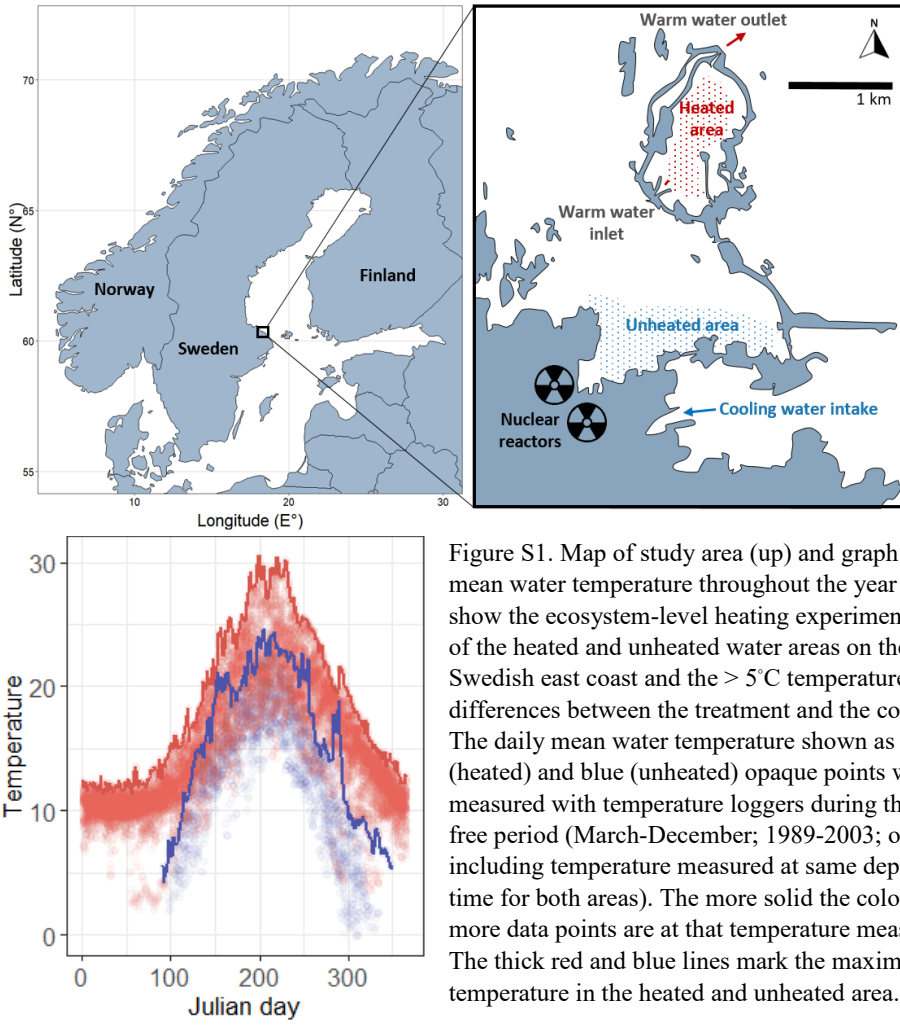

Figure S1. Map of study area (up) and graph of daily mean water temperature throughout the year (down) show the ecosystem-level heating experiment set-up of the heated and unheated water areas on the Swedish east coast and the > 5°C temperature differences between the treatment and the control. The daily mean water temperature shown as red (heated) and blue (unheated) opaque points were measured with temperature loggers during the ice-free period (March-December; 1989-2003; only including temperature measured at same depth and time for both areas). The more solid the color, the more data points are at that temperature measured. The thick red and blue lines mark the maximum temperature in the heated and unheated area.

Comparison of the areas: temperature difference

In winter (December to March), the temperature in the heated area is about 10°C while the unheated area is covered in ice. In the summer months, where the temperature difference is the lowest, it was still minimally about 5°C higher in the heated area (Fig. S1). Temperature in the heated area can exceed 30°C in summer, but in the control area, it never exceeded 25°C (Fig. S1). This temperature difference is unusually large for a natural system (besides exceptional geothermal habitats, e.g. on Iceland) and much larger than the climate change

induced sea surface temperature increase in the Baltic Sea during the same period (up to 1°C, HELCOM 2013). In nature, two areas would usually need to be significantly geographically distant (altitude or latitude) to achieve the same whole-ecosystem temperature difference.

Comparison of the areas: similarities

Except for the large difference (>5°C) in water temperature described in Figure S1, the two areas share many similarities. As they are neighboring, they share the same air temperature, weather conditions and any long-term environmental trends subjected to the whole region, such as climate change. As the heated area was constructed from connecting existing small islands in the archipelago, and the unheated area being part of the same archipelago, they share the general coastal habitat features such as being shallow and near-shore. As water in the heated area has been collected from "Cooling water intake" (Figure S1), pumped through the power plant to absorb heat and then exiting into the heated area via pipes, the two areas most likely share the water quality as it is the same water circulating within this 2 km radius area in the same archipelago. Unfortunately, the only available water quality data that was monitored the same way throughout the study period is water transparency (measured as secchi depth), an indication of eutrophication level. Sandström & Karås (2002) showed that the water transparency is approximately the same in both areas ("Ön" is our control area with secchi depth 3-5 meters and "Biotest basin" is our heated area with secchi depth 4-5 meters, Table 1 in Sandström & Karås 2002).

Test-fishing took place in the dotted areas, and perch were sampled using the same method in the heated and the unheated area. The same stationary bottom gillnets were set at the same depth range (2-5 meters deep) and within 100 meters to shore (Thoresson 1992). All other forms of fishing are prohibited in both areas.

Although the probabilistic maturation reaction norm method enabled us to reveal changes in maturation independently from changes in growth (in length), it is possible that environmental factors other than warming can affect perch maturation and reproduction via, for example, body condition (Uusi-Heikkilä et al. 2011). Body condition is highly dependent on resource availability, which is dependent on total resource and competition among individual consumers. Resource availability might have diverged between the areas over time, e.g. in response to the warming. However, we lack data on e.g. food availability to make proper comparison between the areas over our whole study period. Competition might have played a role, although it cannot be adequately quantified without data on both resources and density of competitors (population density). However, difference in perch population density indicated by perch catch per unit effort (CPUE, number of perch net^{-1} $night^{-1}$) between the two areas did not show a clear difference between the heated and unheated period (Huss et al., 2019, supplement Figure S4).

Additionally, there is no differences in fish species composition between the heated and unheated area before and after heating according to a PCO analysis based on all fish species abundance (Huss et al. 2019, supplement Figure S5).

To summarize, the difference in temperature between the two areas is unusually large and standing out among the environmental and ecological similarities that they share. Therefore, the temperature difference is likely to directly or indirectly (e.g. via resources) have contributed to the observed changes in maturation and reproduction in perch between the areas, and especially between the periods.

Data collection
All perch were measured for length at capture. A size-stratified subset was then made according to the size distribution of all captured perch. This subset is designed to represent the natural distribution of size and sex in the population. Individuals in this subset received detailed sampling and measurements including removal of the left operculum bone (for age and individual growth/size at age measurements), body length (to nearest mm), sex, gonad- (to nearest 0.01 g) and body weight (to nearest g). From 1983 to 2003 (excluding July to September), 3156 female and 3135 male perch were sampled in the heated area, and 1693 female and 2448 male perch were sampled in the unheated area.

The more individuals sampled at one age including information on both immature and mature individuals, the more robust the estimated probabilistic maturation reaction norm is. From modelling and simulation work, 100 individuals with presence of both immature and mature individuals are needed at each age of interest (Barot et al. 2014). There were very few age classes that contained immature males, so we focus our analyses solely on the females. We aim to compare individuals that have been exposed to the same conditions, so ideally we would compare individuals between cohorts over time. Because of limited sample size however, we grouped the cohorts into two periods with strictly 7 years apart.

Age determination and growth measurements
Age was determined by counting the winter rings on the left operculum bone of each sampled perch. The method was validated by counting winter year rings on otoliths of a subset of the sampled perch (Thoresson 1996). The operculum shows allometric growth in relation to the length of the fish. It is widely accepted to measure the radius of the operculum and distances between the winter year rings to estimate annual growth of the fish (Le Cren, 1947). Body size (s_a) at each age a was back-calculated using measurements of the distance between each of these rings (r_a), the radius of the operculum (R) and perch length at capture ($s_{capture}$), using $s_a = s_{capture} \cdot (r_a/R)^{0.861}$ (Thoresson 1996).

Maturation status
Sex and gonadal stages were determined by macroscopic examination of perch gonads at capture. Gonad development stages were categorized into stage 1 (undeveloped), 2 (developing), 3 (spawning), 4 (spent) or sometimes 9 (malformed). We classify stage 1 perch as immature and stage 2-4 as mature and excluded malformed individuals and individuals caught during July-September in the analyses. This is because in July to September it is difficult to identify by macroscopic examination whether a gonad is just recently spent from spawning or undeveloped, and therefore hard to classify an individual as immature or mature.

Table S1. Numbers of immature (I) and mature (M) female and male perch at age 1 to 8 sampled from the heated (H) and unheated area (U) from the two time periods: born within 5 years of warming (cohort 1980-1984) and born after multiple generations of warming (cohort 1991-1996). There is almost no immature male perch at any age in the later time period.

Sex	Area	Cohort	Age 1		Age 2		Age 3		Age 4		Age 5		Age 6		Age 7		Age 8	
			I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M
Female ♀	H	1980-1984	145	48	257	243	133	275	118	214	35	125	5	33	1	17	3	8
	U	1980-1984	1	5	63	27	52	51	81	107	9	54	18	30	18	36	5	0
	H	1991-1996	0	0	9	56	6	197	9	182	5	153	2	55	2	29	1	3
	U	1991-1996	0	0	48	1	59	123	15	212	3	138	4	136	2	118	0	0
Male ♂	H	1980-1984	14	167	52	430	36	440	14	344	4	104	0	10	1	2	0	1
	U	1980-1984	0	4	20	59	7	57	20	140	12	2	6	4	6	6	0	0
	H	1991-1996	0	0	0	144	0	431	0	244	0	219	0	27	0	16	0	0
	U	1991-1996	0	0	1	68	1	484	0	581	0	324	0	306	0	181	0	0

Table S2. Results (AIC score, Nagelkerke's R2 and sample size) of different ogive models describing perch maturation status in the heated and unheated area. We assume maturation status and perch age (a), size (s) and/or the interaction between them fit a logistic regression. To capture the changes over time, perch maturation status are described separately for each period consisting of cohorts 1980-1984, and 1991-1996, including ages where both immature and mature individuals were sampled**.**

Area	Cohorts	Age	Formula	AIC	Nagelkerke's R2	N
Heated	1980-1984	1-8	m ~ a * s	2017	0.19	1660
			m ~ a + s	2046	0.17	1660
			m ~ s	2047	0.16	1660
			m ~ a	2112	0.12	1660
	1991-1996	2-8	m ~ a * s	250	0.13	709
			m ~ a + s	259	0.08	709
			m ~ a	276	< 0.01	709
			m ~ s	262	0.07	709
Unheated	1980-1984	1-7	m ~ a * s	716	0.11	552
			m ~ a + s	714	0.11	552
			m ~ s	713	0.11	552
			m ~ a	733	0.06	552
	1991-1996	2-7	m ~ a * s	369	0.61	859
			m ~ a + s	407	0.56	859
			m ~ s	407	0.56	859
			m ~ a	482	0.45	859

Gonado-somatic index

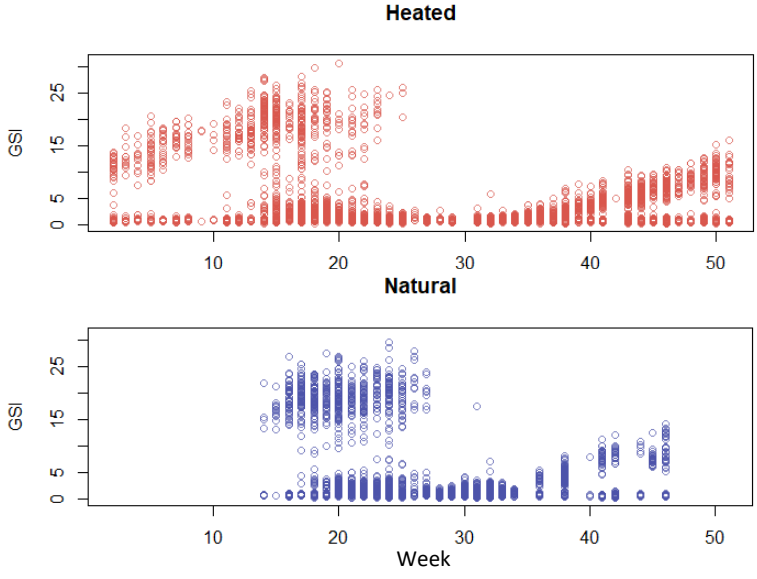

Figure S2. Gonado-somatic index (GSI) distribution of perch from the two areas year-round (1980-1984 and 1991-1996), showing the gradual development from spent gonads around week 30, gonads starting to develop in autumn to pre-spawning and spawning. GSI values close to zero all year round indicate presence of juveniles. Although we do not have extensive data, perch in the heated area seem to have an earlier spawning season than perch in the natural temperature area. It has been documented (Lukšienė et al. 2000) and observed (Gårdmark & Huss, personal communication, February 1, 2022) that perch spawning season differs between the heated and non-heated area, being March-May in the heated area and late April to early June in the non-heated area.

Gonad weight and gonadal status were sampled among a fewer sampling years (1984-1990, 1994, 1996 and 1998) than age and annual growth, so there is less data available for gonad analysis comparing to the probabilistic maturation reaction norm (PMRN) analysis.

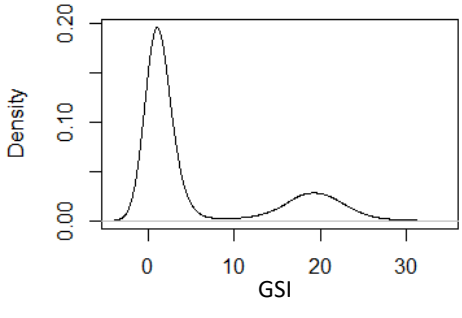

Figure S3. Density distribution of gonado-somatic index (GSI) from week 10 to 30 of perch from the two study areas from 1980-1998 (all sample years). It is bimodal, which indicates that during this period there are two different stages gonads present – spawning and any other stage with low gonad mass. The spawning individuals have an average GSI ≈ 20%, similar to previously documented for female perch (Le Cren 1951).

Table S3. Number of 2- to 5-year-old perch with GSI >10 or GSI <10 sampled and used (only GSI > 10) for the analysis of reproductive investment. This shows that there were few data for 2-year-old perch in both areas and periods and for 3-year-olds in the natural area in the early period. Thus, we only included 3-5-year-old perch with GSI >10 in our analysis.

Area	Cohort	Age 2		Age 3		Age 4		Age 5	
		>10	<10	>10	<10	>10	<10	>10	<10
Heated	1980-1984	7	150	47	170	52	160	25	56
Natural	1980-1984	0	49	1	70	18	171	13	99
Heated	1991-1996	3	80	39	199	48	159	46	118
Natural	1991-1996	0	70	27	209	66	204	68	112

Maturity ogive models and effects on PMRN

The best ogive model was $o \sim \alpha_0 + \alpha_1 a + \alpha_2 s + \alpha_3 a * s$ (Figure S4), except for cohorts 1980-1984 in the natural temperature area, for which the best model was $o \sim \alpha_0 + \alpha_1 a + \alpha_2 s$ (Table S2, see above, but as shown in Figure S5 there is little difference between these alternative models). To allow for comparisons between areas and periods, we used the model $o \sim \alpha_0 + \alpha_1 a + \alpha_2 s + \alpha_3 a * s$ throughout to predict maturity ogives and subsequently calculating probabilistic maturation reaction norms.

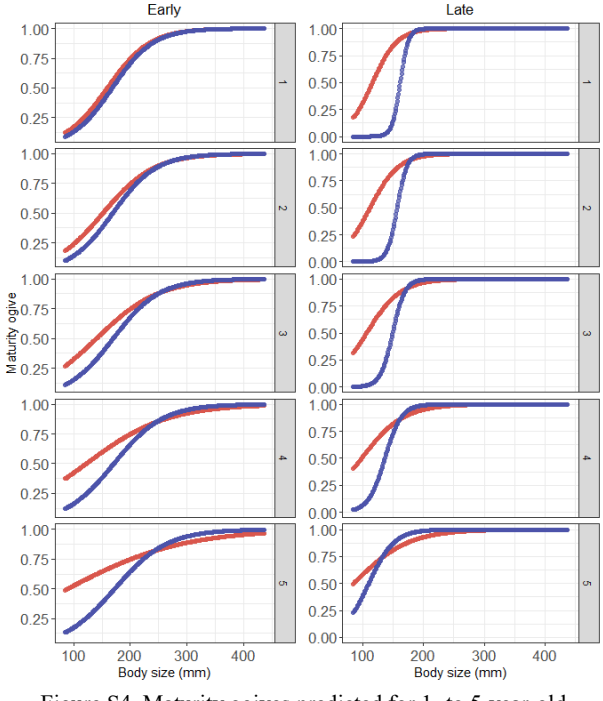

Figure S4. Maturity ogives predicted for 1- to 5-year-old perch sampled in the heated area (red) and natural temperature area (blue) with body size from 85 to 435 mm (same range as the sampled perch) using the selected ogive model $o \sim \alpha_0 + \alpha_1 a + \alpha_2 s + \alpha_3 a * s$ (Table S2).

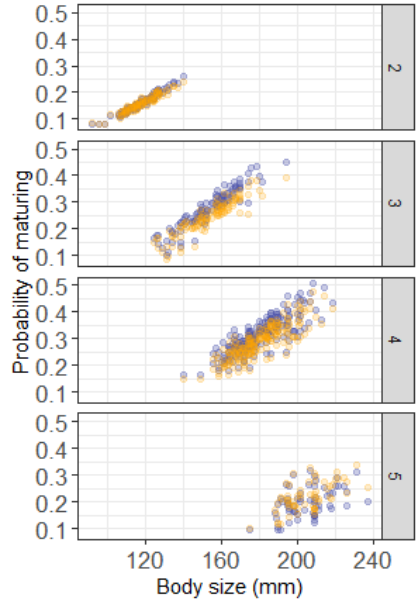

Figure S5. Probabilistic maturation reaction norms of cohorts 1980-1984 in the unheated area showing that probabilities of maturing for each age (2-5 year-old) calculated from different (the first two) ogive models (Table S2) in this case are very similar (the blue and orange dots). Our findings are not affected by this.

Model assumptions of the logistic regression (Stoltzfus, 2011) are 1) independent errors, no duplicately measured responses, which is met since every perch was an independent individual; 2) the relationship between the continuous variables and their logit-transformed outcomes should be linear, which is met (Figure S6); 3) absence of multi-collinearity or redundancy among independent variables, but this is often tolerated if the variables are of great biological interests, which is our case for perch age and size; 4) no strongly influential outliers, which was checked for and not found (Figure S7).

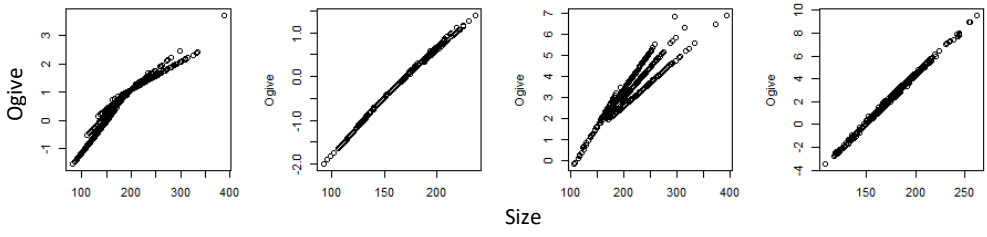

Figure S6. Diagnostic plot showing the only continuous variable "size" are associated with the logit-transformed ogive (per age) for the best ogive models per area per period (Table S2). Shown from left to right is early and late period in the heated area and the two periods in the unheated area.

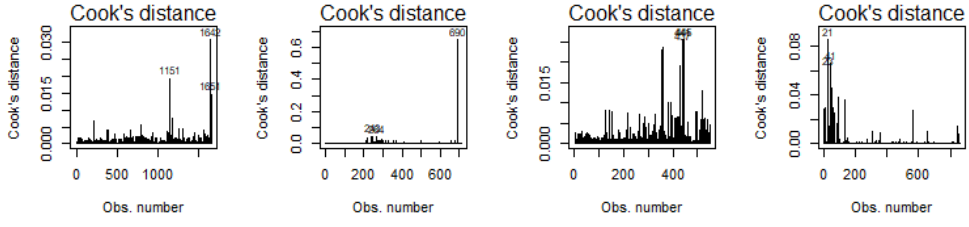

Figure S7. Diagnostic plot showing there is no influential outlier for the best ogive models per area per period (Table S2). Shown from left to right is early and late period in the heated area and the two periods in the unheated area.

Model selection and validation of probabilistic maturation reaction norms (PMRN) and gonado-somatic index (GSI)

For both probability of maturing $m(a, s)$ and GSI, we ran the null and full model as a function of only age, size and age*size, and as a function of age, size, area, period and all possible interactions, respectively.

We selected the best models manually stepwise and backwards starting from the full model. Starting from the full model, we in each step removed one parameter from the model, starting from the highest order of interaction and compared the AIC between the new and previous model. Age, size and age*size were never removed. If the new model AIC was lower than that of the previous model, we removed the second-highest order of interactions left in the new model and thus created another new model. If the AIC was not lower, we added the removed parameter back and removed another one from the same order of interactions or one from one order of interaction lower if none was left in the same order. We continued these two steps until the new model AIC stopped decreasing. We consider the best model to be the one with the lowest AIC, and all models within two units of AIC from it as indistinguishable.

We predicted $m(a,s)$ using the best model for the PMRN. We compared the prediction with original data and they align well (Figure S8).

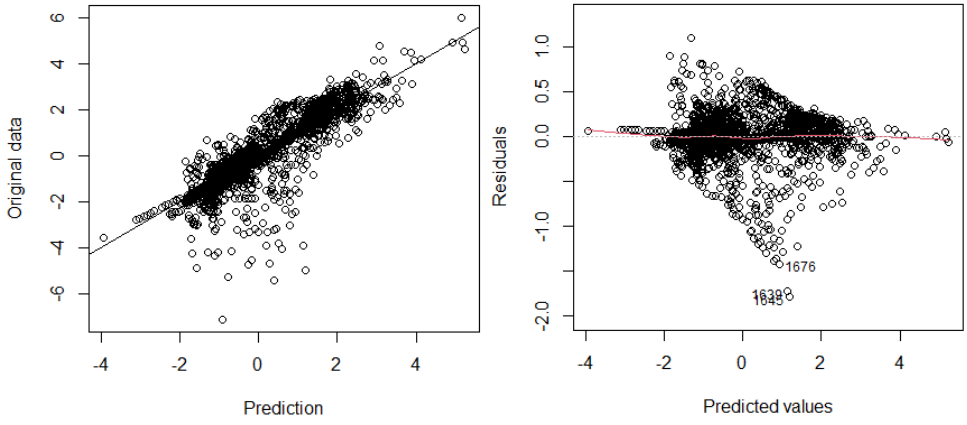

Figure S8. Diagnostic plots for PMRN model validation. Left: logit transformed original probability of maturation *m(a,s)* vs predicted *m(a,s)* shows that they align up. Right: the residuals vs fitted values.

Table S4. Coefficients of the parameters of the best probabilistic maturation reaction norm model (see Table 1 for model selection results).

	Estimate	Std.	Error	z value	Pr(>|z|)
(Intercept)	-5.02E+00	7.18E-01	-6.99	2.74E-12	***
Age	5.99E-01	2.25E-01	2.669	0.00761	**
Size	3.60E-02	5.05E-03	7.134	9.73E-13	***
period(late)	2.69E+00	6.23E-01	4.322	1.55E-05	***
area(control)	-6.57E-01	3.29E-01	-1.999	0.04563	*
age:size	-6.41E-03	1.26E-03	-5.102	3.36E-07	***
periodlate:area(control)	-1.70E+01	3.78E+00	-4.501	6.75E-06	***
age:period(late)	-8.32E-01	3.44E-01	-2.418	0.0156	*
age:size:area(control)	7.09E-04	5.01E-04	1.414	0.15729	
age:size:period(late)	1.09E-03	1.06E-03	1.03	0.30295	
age:period(late):area(control)	3.29E+00	1.05E+00	3.125	0.00178	**
size:period(late):area(control)	9.53E-02	2.36E-02	4.042	5.29E-05	***

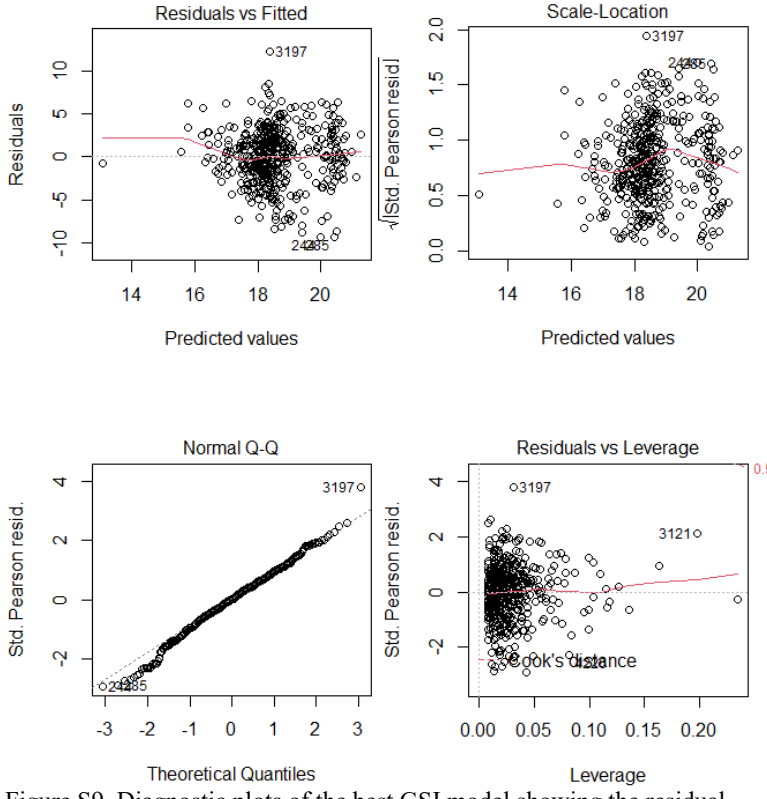

Figure S9. Diagnostic plots of the best GSI model showing the residual pattern is more or less normal.

Results: Differences between heated and unheated areas

Differences in probabilistic maturation reaction norms between the two areas were tested with Mann-Whitney U tests (Table S5). Differences in mean size at 50% probability of maturation (Lp50, predicted from the best PMRN model) in the heated area between the early and the late study period is shown in Figure S10, and differences in variation (residual sum of squares) between the periods in each of the areas in Figure S11.

Differences in reproductive investment (GSI) between the areas, and how it changes between the study periods, are shown in Figure S12 and were tested using Student's t-tests (Table S6).

Potential alternative explanations to the ones in the main text for these differences are discussed below, including shifts in the size-classes of perch sampled (Fig. S13) and sampling months in the two study periods (Fig. S14).

Table S5. Mann-Whitney U pairwise test results, to test if probability of maturing differs between area or period (per age). Effect size is calculated as $\eta^2 = Z^2/n$ and indicates the percentage of variance in the dependent variable explained by the independent variable, where Z is reported from the MW U test (Z = qnorm(p-value)) and n is the total sample size (Fritz et al. 2012).

Test pair	Test assumption	P	η^2 (one-sided)	n
Age 2, area, early	Heated > natural	< 0.01	0.30	590
Age 3, area, early	Heated > natural	< 0.01	0.08	511
Age 4, area, early	Heated > natural	< 0.01	0.05	520
Age 5, area, early	Heated > natural	< 0.01	0.03	220
Age 2, heated, period	Early < late	< 0.01	0.30	565
Age 3, heated, period	Early < late	< 0.01	0.39	611
Age 4, heated, period	Early < late	< 0.01	0.04	489
Age 5, heated, period	Early < late	0.13	0.01	247
Age 2, unheated, period	Early < late	0.98	0.03	139
Age 3, unheated, period	Early < late	< 0.01	0.37	285
Age 4, unheated, period	Early < late	< 0.01	0.70	415
Age 5, unheated, period	Early < late	< 0.01	0.64	204
Age 2, area, late	Heated > natural	< 0.01	0.73	114
Age 3, area, late	Heated > natural	< 0.01	0.10	385
Age 4, area, late	Heated > natural	1	NA	384
Age 5, area, late	Heated > natural	1	NA	231

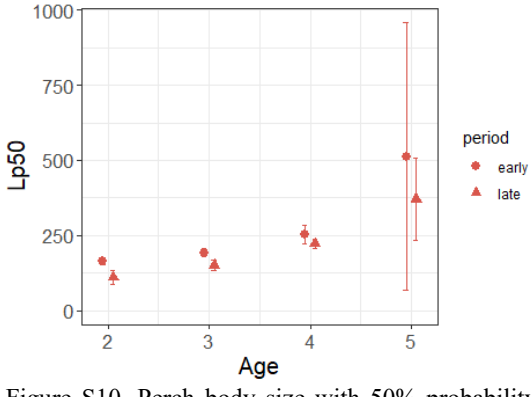

Figure S10. Perch body size with 50% probability of maturing (Lp50) for each age 2-5, predicted by the best model but only including the heated area for the early (circle) and late (triangle) period and the 95% confidence interval bar. Perch from the late period generally mature at smaller size (have lower Lp50) than perch from the early period, especially for age 2 and 3 for which the CI are not overlapped.

Another explanation in addition to the ones in the main text (alternative evolutionarily stable strategies; food condition) for the bigger variance in maturation in the heated area (as evident in Figure S11) could be that maturation size likely has a hump-shaped relationship with temperature and that the water temperature in the heated area have exceeded perch optimal temperatures in some years (e.g. > 30 °C, Figure S1; cf. Huss et al. 2019). Temperatures above optimal are especially likely to be the case optimal for large sized individuals (Huss et al. 2019), as optimum temperature for body growth decreases with size (Lindmark et al. 2022). Too high temperature can potentially postpone maturation (Luksiene and Sandström 1994; Dhillon and Fox 2004; Miranda et al. 2013; Shahjahan, Kitahashi, and Ando 2017), which would mean increased size at maturation for first-time spawners. The frequency of extreme temperatures can vary between years, leading to some cohorts increasing size at

maturation, which could contribute to the observed increased variance in maturation size in the later cohorts in the heated area. Alternatively, changes in food condition in relation to temperature and variation in diet among individuals (Svensson et al. 2017; Scharnweber and Gårdmark 2020) may have led to variation in food-dependent maturation. Because of the potential opposite direction of responses to warming depending on its extent, care should be taken when extrapolating our findings on effects of warming beyond the thermal regimes in our system and to other organisms.

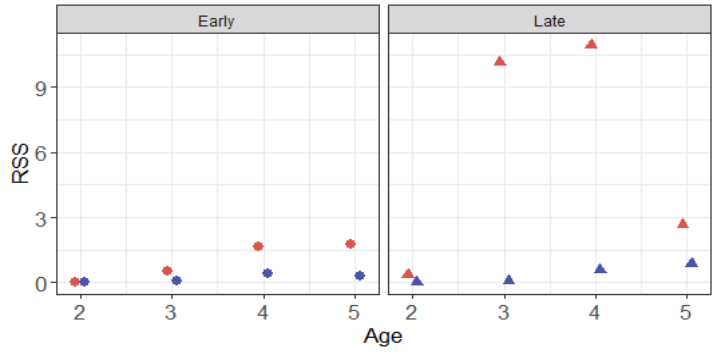

Figure S11. We calculated residual sum of squares, RSS = (m(a,s) - $m_{predicted}$), as a proxy for variance in probability of maturing for 2-5-year-old perch from the heated (red) and unheated (blue) area (m is predicted from the best model in Table 1). Variance is substantially larger in the heated area than the natural temperature area, except for 2-year-old perch from the early period. For both periods, 2-year-old perch have the lowest variance. In the early period, variance increased over age. In the late period, variance is very big for 3-5-year-old perch.

Table S6. Coefficients of the parameters of the best GSI model (see Table 2 for model selection results).

	Estimate	Std.	Error	t value	Pr(>|t|)	
(Intercept)	-20.3885	8.621073	-2.365	0.018468	*
age	9.766454	2.352479	4.152	3.97E-05	***
size	0.214683	0.046361	4.631	4.81E-06	***
area(control)	-18.9215	5.267484	-3.592	0.000365	***
period(late)	52.72782	12.82038	4.113	4.67E-05	***
age:size	-0.05147	0.011788	-4.366	1.58E-05	***
age:area(control)	6.345855	2.483757	2.555	0.010958	*
age:period(late)	-12.3293	3.241408	-3.804	0.000163	***
size:period(late)	-0.2773	0.063418	-4.373	1.54E-05	***
age:size:area(control)	-0.01312	0.007099	-1.849	0.065165	.
age:size:period(late)	0.062896	0.015423	4.078	5.40E-05	***
age:area(control):period(late)	-3.15795	1.4018	-2.253	0.024768	*
size:area(control):period(late)	0.081914	0.030916	2.65	0.008352	**

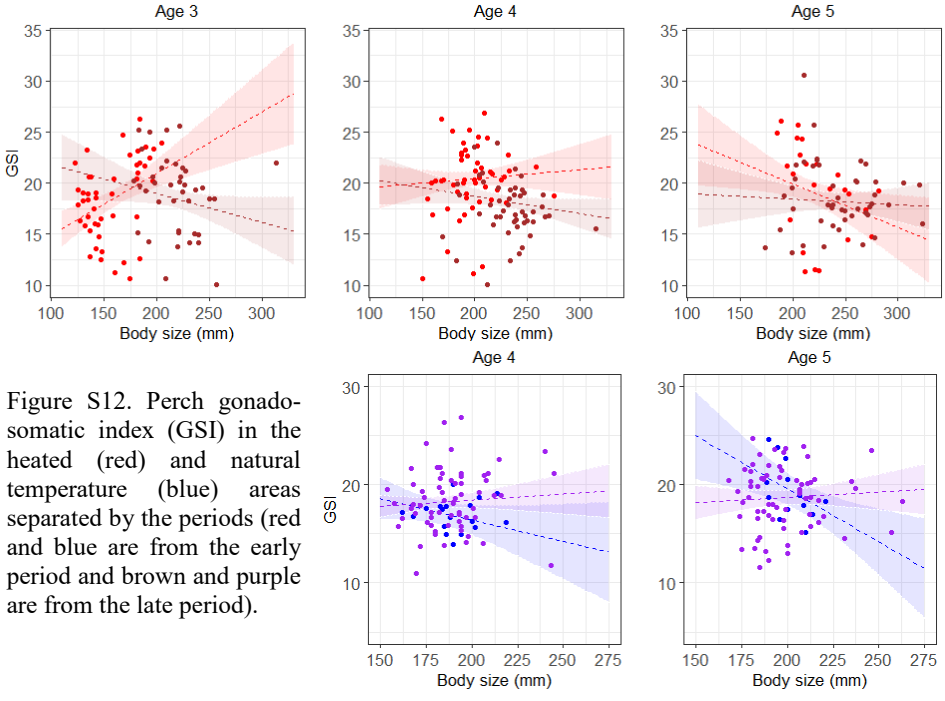

Figure S12. Perch gonado-somatic index (GSI) in the heated (red) and natural temperature (blue) areas separated by the periods (red and blue are from the early period and brown and purple are from the late period).

Table S7. Results of Student T tests, testing if there is difference on GSI between area and period (per age). Shown are p-values and effect sizes estimated as Cohen'd index = [mean(group1)-mean(group2)]/sd(group1 and group2). It shows that the bigger GSI in heated area only applies in the early period.

	P	Cohen's d	N1	N2
Age 4, area, early	< 0.01	1.05	52	18
Age 5, area, early	0.57	/	25	13
Age 4, area, late	0.96	/	48	66
Age 5, area, late	0.54	/	46	68
Age 4, heated, period	< 0.01	0.86	52	48

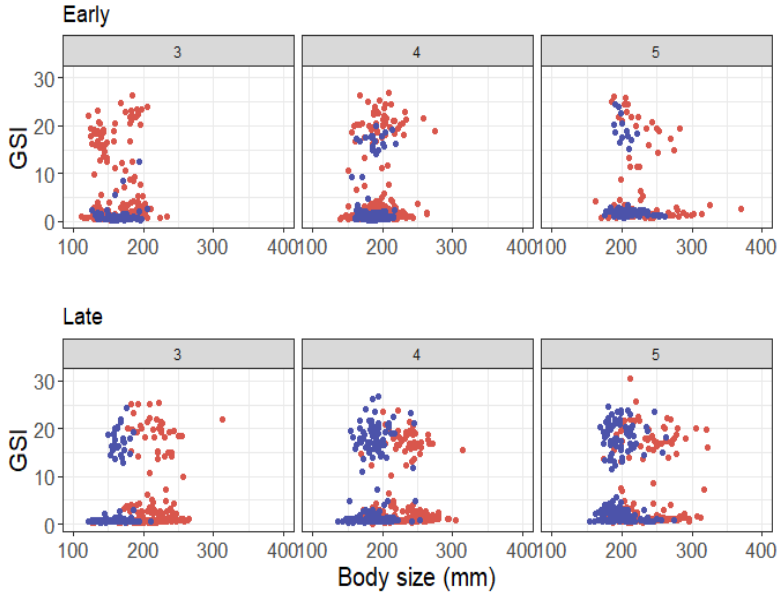

Figure S13. Individual GSI versus body length in heated (red) and unheated (blue) area per age from age 3 to 5 of perch sampled for GSI analysis, showing that fish sampled in the heated area have a larger range of body size than fish in the unheated area with natural temperatures for old perch in the early period (top row) and in general for the late period (bottom row). This is especially the case for perch that were close to spawning (GSI > 10%), which may have limited our ability to find differences in GSI between the two areas.

Over time, it is highly likely that differences among other environmental factors between the areas are developing. For example, food conditions might have changed over time. Biomass of benthic invertebrates, an important diet item of perch (Svensson et al., 2017), initially increased with warming, but collapsed in 1989 (Sandström 1991), right before the late period in our study system. This could have adversely affected perch body condition, leading to a delayed maturation.

There are some additional limitations to consider in the study. For example, because sampling months varied over time (Figure S14) and perch age was determined by the number of winter rings on their operculum bones, some perch could have lived one more growth season than their same-aged peers (i.e. assigned the same age in years). This might have biased the maturity ogive calculation. However, our growth estimates were made from back-calculated growth of each individual, and variation in sampling month will therefore not affect the PMRN estimations.

When calculating m(a,s), we predicted ogives (o(a,s)) outside the input data range, for example when a = 1 year for the late period in both areas, which could have made the

prediction for m(a,s) less reliable for 2-year-old perch. GSI as a measure of gonad development provides no information about detailed reproductive attributes, e.g. quality and quantity of the eggs or indications of future resorption of gonads. Thus, inferences about consequences of observed changes in GSI for reproductive success should be avoided.

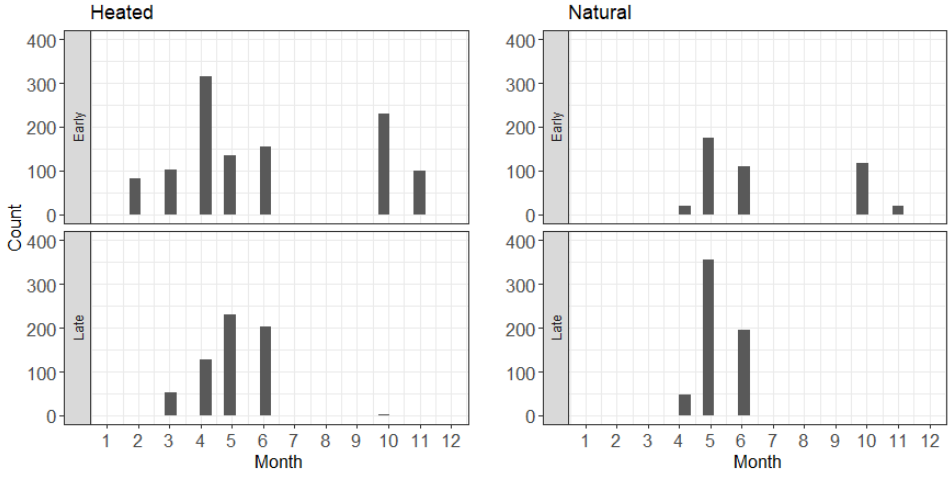

Figure S14. The number of perch sampled from the two areas during the two periods throughout the year (July – September were excluded) shows that in the early period, perch were sampled almost year round. In the late period, however, perch were only sampled from March to June. Since age of each individual perch was determined as the number of winter rings on their opercula regardless of the sampling month, perch consequently would end up having the same age even if one is sampled in October and have lived one more growth season than one sampled in April.

PMRN and GSI model selection lists

Table S8. Full list of model results for selection of variables explaining probability of maturing for perch aged 2-5 years across both areas (heated vs unheated) and periods (early 1980-1984 and late 1991-1996). Null model (without effects of area or period), full model (effects of age, size, area and period and all their interactions) and the best model based on lowest AIC are marked.

	Formula	AIC	Residual deviance
Full	m_as ~ age * size * area * period	2152	150
Null	m_as ~ age * size	3301	619
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + age:area:period + size:area:period	2164	154
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + age:area:period + size:area:period + age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + size:area:period+ age:size:period:area	2169	157
	age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + age:area:period + age:size:period:area	2180	159
	age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + age:area:period + size:area:period	2167	157
	age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:area:period + size:area:period	2160	154
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + size:area:period+ age:size:period:area	2169	157
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:area:period+ age:size:period:area	2180	159
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + size:area:period+ age:size:period:area	2169	157
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + area:area:period+ age:size:period:area	2178	160
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:area:period + size:area:period	2164	158
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + size:area:period+ age:size:period:area	2169	157

	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:area:period + age:size:period:area	2180	159
	m_as ~ age + size + area + period + age:area + age:period + size:area + size:period + area:period + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:period + size:area + size:period + area:period + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:area + size:area + size:period + area:period + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:period + area:period + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + area:period + age:area:period + size:area:period+ age:size:period:area,	2152	150
	m_as ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + age:area:period + size:area:period+ age:size:period:area	2189	164
	m_as ~ age + size + area + period + age:size + age:period + area:period + age:area:period + size:area:period+ age:size:period:area	2152	150
	m_as ~ age + size + age:size + area + period + age:period + age:area + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2151	151
	m_as ~ age + size + age:size + area + period + size:period + age:area + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2152	150
	m_as ~ age + size + age:size + area + period + size:period + age:period + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2150	150
	m_as ~ age + size + age:size + area + period + size:period + age:period + period:area + age:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2147	151
	m_as ~ age + size + age:size + area + period + size:period + age:period + size:area + age:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2189	164
	m_as ~ age + size + age:size + area + period + size:period + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2150	150
	m_as ~ age + size + age:size + area + period + size:period + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2151	151
	m_as ~ age + size + age:size + area + period + size:period + age:area + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2147	151

	m_as ~ age + size + age:size + area + period + age:period + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2149	152
	m_as ~ age + size + age:size + area + period + size:period + age:period + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2145	151
	m_as ~ age + size + age:size + area + period + age:period + age:area + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2145	153
	m_as ~ age + size + age:size + area + period + period:area + size:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2149	152
	m_as ~ age + size + age:size + area + period + size:period + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2149	152
	m_as ~ age + size + age:size + area + period + size:period + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2145	153
	m_as ~ age + size + age:size + area + period + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2143	153
Best	m_as ~ age + size + age:size + area + period + age:period + period:area + age:size:area + age:period:area + age:period:size + size:period:area + age:size:period:area	2143	153

Table S9. Full list of model results for selecting variables explaining the building up of gonads of perch aged 3-5 years, across both areas (heated vs unheated) and time periods (early 1980-1984 and late 1991-1996).

	Formula	**AIC**	**Residual deviance**
Full	GSI ~ area*age*size*period	2362	4652
Null	GSI~age + size + age:size	2383	5134
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + age:area:period + size:area:period	2360	4658
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + size:area:period	2363	4701
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + age:area:period + size:area:period	2362	4687
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:area:period + size:area:period	2377	4846

	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + age:area:period	2360	4672
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + area:period + age:size:period + age:area:period	2360	4712
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + area:period + age:size:period	2360	4738
	GSI ~ age + size + period + age:size + size:area + period:area +age:size:area + period:area:age + period:area:size + age:size:period:area	2360	4668
	GSI ~ age + size + age:size + size:area + period:area + age:size:area + period:area:age + period:area:size + age:size:period:area	2360	4668
Best	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:area + age:size:period + age:area:period + size:area:period	2358	4672
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:area + age:size:period + size:area:period + age:size:area:period	2359	4681
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:period + age:area:period + size:area:period	2360	4688
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:area + age:size:period + age:area:period + size:area:period + age:size:area:period	2360	4670
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:area + age:area:period + size:area:period + age:size:area:period	2360	4670
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + age:area:period	2361	4699
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period + age:size:area:period	2362	4691
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + age:size:period	2361	4703
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area + size:area:period	2381	4920
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period + size:area:period	2363	4725
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period	2361	4727
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + size:area:period	2380	4921
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:area	2380	4924
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period	2378	4924
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:area + size:period + area:period + age:size:period:area	2362	4691
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + area:period + age:size:period	2360	4737
	GSI ~ age + size + area + period + size:area + age:area + age:period + size:period + area:period + age:size:period	2361	4727
	GSI ~ age + size + area + period + age:size + size:area + age:period + size:period + area:period + age:size:period	2364	4775
	GSI ~ age + size + area + period + age:size + age:area + size:area + size:period + area:period + age:size:period	2378	4925

	GSI ~ age + size + area + period + age:size + age:area + size:area + age:period + area:period + age:size:period	2379	4933
	GSI ~ age + size + area + period + age:size + age:area + size:period + age:period + size:area + age:size:period	2367	4806
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + area:period	2377	4935
	GSI ~ age + size + area + period + age:size + age:area + size:period + area:period + age:size:period	2377	4937
	GSI ~ age + size + area + period + age:size + age:period + size:period + area:period + age:size:period	2366	4815
	GSI ~ age + size + area + period + age:size + age:area + age:period + area:period + age:size:period	2377	4939
	GSI ~ age + size + area + period + age:size + age:area + age:period + size:period + age:size:period	2367	4828
	GSI ~ age + size + area + period + age:size + age:area + size:period + area:period	2375	4938
	GSI ~ age + size + area + period + age:size + size:period + area:period + age:size:period	2375	4943
	GSI ~ age + size + area + period + age:size + age:area + area:period + age:size:period	2376	4945
	GSI ~ age + size + area + period + age:size + age:area + size:period + age:size:period	2379	4982
	GSI ~ size + area + period + age:size + age:area + size:period + area:period + age:size:period	2378	4967
	GSI ~ age + area + period + age:size + age:area + size:period + area:period + age:size:period	2379	4980
	GSI ~ age + size + period + age:size + age:area + size:period + area:period + age:size:period	2377	4965
	GSI ~ age + size + area + age:size + age:area + size:period + area:period + age:size:period	2375	4940

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Multi-decadal warming alters predator's effect on prey community composition

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Predator responses to warming can occur via phenotypic plasticity, evolutionary adaptation or a combination of both, changing their top-down effects on prey communities. However, we lack evidence of how warming-induced evolutionary changes in predators may influence natural food webs. Here, we ask whether wild fish subject to warming across multiple generations differ in their impacts on prey communities compared with their nearby conspecifics experiencing a natural thermal regime. We carried out a common garden mesocosm experiment with larval perch (*Perca fluviatilis*), originating from a heated or reference coastal environment, feeding on zooplankton communities under a gradient of experimental temperatures. Overall, in the presence of fish of heated origin, zooplankton abundance was higher and did not change with experimental warming, whereas in the presence of fish of unheated origin, it declined with experimental temperature. Responses in zooplankton taxonomic and size composition suggest that larvae of heated origin consume more large-sized taxa as the temperature increases. Our findings show that differences between fish populations, potentially representing adaptation to their long-term thermal environments, can affect the abundance, biomass, size and species composition of their prey communities. This suggests that rapid microevolution in predators to ongoing climate warming might have indirect cross-generational ecological consequences propagating through food webs.

1. Introduction

Organisms respond to prevailing climate warming by means of plasticity during acclimatization and evolutionary changes over generations, or both [1]. These responses can lead to physiological and life-history trait changes in organisms [1,2]. Despite extensive efforts in investigating species [3] and community responses to warming [4,5], such studies are rarely carried out over the span of organisms' multiple generations [2,6–9]. When studies do span multiple generations, they are rarely field-based but most often carried out under laboratory conditions that are hard to extrapolate to responses in nature [10,11], or are using space for time substitution approaches [12]. While the latter often achieve large thermal gradients, there are inevitably other biotic and abiotic differences brought by the spatial gradients that can confound the effect of differences in temperature [12]. Yet, given fast ongoing environmental changes owing to global warming, there is a pressing need to investigate the cascading effects of potential evolutionary adaptations to

warming in natural predators on prey communities, to improve our ability to predict the impacts of global change on food web stability and ecosystem functioning [13].

In aquatic systems, fish commonly exert top-down control on zooplankton communities by size-specific feeding [14]. Microevolutionary adaptation or acclimatization to warming in fish physiology, life-history traits and behaviour may affect their feeding, for example via changes in their metabolism, growth, development, morphology and body size [15–18]. Metabolic changes under warming can have contrasting effects on feeding. An increased top-down effect is predicted to occur owing to an increase in metabolic rate, which is common in warmer environments [19]. This requires and also enables organisms to consume more prey or prey of larger sizes [20]. However, if they are not able to fully compensate by increasing their feeding rate, increased metabolic costs can result in lower net energetic gains in warmer environments, with less energy being allocated to activities such as growth, locomotion and even motivation to initiate predation [21,22], which could lead to a decrease in top-down effect. However, a compensatory metabolic response (and potentially adaptive) in the form of a depression in standard metabolic rate (SMR) has been found in several warm-acclimatized or warm-adapted fish species [16,23–25], probably to reduce the energy loss in a warmer environment. In such cases, individuals require less food to maintain their energy balances. Their top-down effect on prey may then be lowered. Physiological adaptation can also lead to faster development [26] or body growth [11] in warm environments. Because organisms then get a larger body size when young [15] and thus potentially a larger gape size [27], or a more robust body at a given age, this may enable them to switch their diet towards larger or better swimming prey at an earlier age compared with individuals with slower growth [11,28,29]. This might lead to differences in prey selection reflected by size- or species-dependent top-down effect or phenological change in the top-down effect and thus in its strength at a given time in the season.

Effects of adaptation or acclimatization on traits or processes that can affect feeding can play out differently depending on the extent of temperature change relative to their natal habitat temperature, and exposure time. For example, thermal tolerance or SMR often follow a hump-shaped function of the novel environmental temperature [16,30]. Indirect effects from these responses on predator top-down effects could therefore also be hump-shaped, which has been shown for attack and maximum ingestion rates [6,31–33]. Changes in top–down effects on prey communities from warming-induced responses in predators may thus not be uni-directional across temperatures.

While there is evidence for thermal adaptation in predators [16] and that thermal acclimatization in predators can shape predator–prey interactions [6,8], we still lack experimental tests of how cross-generational responses of predators to warming may influence prey community composition under different temperatures. This is an important question bridging direct microevolutionary responses in predators and ecological responses in the food web within the eco–evo feedback loop triggered by warming. Such feedbacks have been found in some aquatic predators [12], but responses in prey communities to indirect effects of multi-generational warming via trophic interactions are yet to be demonstrated in fish. Compared to no warming, the warming-affected top-down effect can lead to less or more biomass reduction, shifts in species composition and size distribution of their prey [34], as well as behavioural and morphological variations in prey [35]. Warming-induced shifts in relative size distribution or turnover rates can also result in predator–prey mismatches [28]. Food web dynamics may, therefore, change differently if prey responds to warming adaptation in predators, and may then also cascade to lower trophic levels and affect the response of ecosystem processes to warming [36,37].

Here, we investigate whether the thermal origin of larval fish affects their zooplankton prey communities under a warming gradient, through a common garden mesocosm experiment. We used fish larvae that originated from two adjacent wild populations: one with ambient temperature (hereafter unheated-origin population, UN) and the other heated for many generations (heated-origin population, H). During the experiment, fish larvae of both origins fed on one zooplankton community from the unheated area. Predators and prey were jointly exposed to a gradient of experimental temperatures, and direct measures on all trophic levels were taken. This allowed us to study the ecological consequences of warming-induced long-term responses in wild fish populations on lower trophic levels. Our findings imply that impacts of climate warming on predators across generations can have indirect, yet substantial, effects on their prey communities via shifts in trophic interactions.

2. Material and methods

(a) Thermal origin of fish larvae

The larval Eurasian perch (*Perca fluviatilis*, hereafter perch) used in the experiment originated from parental populations from two areas: (i) an area subjected to artificial heating for 41 years and (ii) an adjacent coastal area experiencing a natural temperature regime. The heated population has been residing in a 1 km^2 heated area since 1980 and subjected to a temperature of 5–10°C above the natural, caused by heated water discharge from nearby nuclear reactors on the western Baltic Sea coast (60°43′ N 18°19′ E, figure 1*a*). The design of the set-up ensured that the two populations were exposed to similar environmental conditions, other than the heated water flow-through (for shared coastal environmental characteristics and water properties, see electronic supplementary material, table S1). A physical barrier to the exchange of fish between the heated and unheated areas was in place shortly after the enclosure construction of the heated area, resulting in a 23 year isolation of perch on there from the surrounding area. It was a 15 mm mesh size metal grid at the outlet of the heated area blocking the only narrow passage from the heated area to the surrounding coast (figure 1*a*). Plenty of studies have revealed differences in perch life-history traits and physiology in the heated area compared with the unheated area over the decades ([14,17,24,36–40]; also see electronic supplementary material, Perch larvae thermal origin: study population). Analyses using less than 10 microsatellite loci in earlier studies show significant genetic differentiation between the heated and the unheated population [38,41]. The grid

was removed in 2004, which increased the probability of larger fish (>10 cm [39]) dispersing between the areas. Nevertheless, the 23 year isolation and the continuing semi-isolation of the two populations since 2004 still result in some separation between the two populations [38,41]. The combination of phenotypic trait changes and genetic shifts suggests possible warming-induced microevolution in perch from the heated area.

To study the effects on prey communities of larval perch from these two geographically close but thermally contrasting habitats, we collected 15 separate roe strands from each area on 10 May 2021, representing the H and UN perch population (electronic supplementary material, table S2). As available genetic evidence of differentiation between the two populations is limited, especially for times after the grid removal [38,41], we conducted a simple genetic analysis on 14 microsatellite loci in non-coding regions using all the 15 roe strands (as described in electronic supplementary material, Genetic analysis) to test whether these two populations are indeed separated. It showed low, but statistically significant genetic differentiation between the heated and unheated populations (electronic supplementary material, Genetic analysis). Although this is not enough to demonstrate any selection signature owing to warming or any consequent adaptation to warming, it indicates that the sampled roe strands come from two genetically differentiated populations—a precondition for our experiment.

For hatching larvae to use in the experiment, we selected eight roe strands with similar width and egg adhesion out of the 15 roe strands collected from each habitat and transferred to indoor aquaria. Each roe strand was placed in separate aquaria with approximately 100 l aerated, unfiltered coastal water with a 16 : 8 h light : dark photoperiod indoors at room temperature. The remaining seven roe strands from each population were kept in smaller aquaria (approx. 40 l) as backups (electronic supplementary material, table S2). Perch larvae started hatching from 17 May (for records of hatching status, see Record_hatchingstatus.xlsx), and on 22 May, there was an adequate amount of newly hatched larvae (<5 days old) to start the experiment naming 22 May as experiment day 0. The 10 individual perch larvae introduced in every fish-present (H and UN) mesocosm consisted of two individuals each from five selected roe strands of each of the heated and unheated origin (electronic supplementary material, table S2). These five roe strands were selected such that most larvae hatched around the same time to better control for other effects than their population of origin. At the start of the experiment, we acclimatized the fish larvae to the experimental mesocosm by gradually lowering and immersing a bag containing aquaria water and larvae into the mesocosm water.

(b) Mesocosm experiment

We established 38 outdoor mesocosms using tanks (FlexiTank, Max Grow Shop) of a volume of approximately 400 l, diameter of 0.68 m, height of approximately 1.1 m, made of polyurethane fabric and supported by rods. Altogether, 26 of these tanks were inoculated with 10 fish larvae each (13 tanks with heated-origin and 13 with unheated-origin fish larvae) and the remaining 12 tanks were kept without fish as control (figure 1*b*). Hereafter, we refer to these as heated-origin mesocosms (H), unheated-origin mesocosms (UN) and no fish mesocosms (NF). H and UN mesocosms are jointly referred to as fish-present mesocosms. The experiment was conducted outdoors for 21 days (22 May–11 June 2021, experiment day 0–20). In these open-to-atmosphere tanks, we generated a simplified pelagic food web with plankton and fish larvae. The mesocosms were filled to have an approximately 95 cm water column with 350 l water from the unheated area (salinity approx. 5 PSU) filtered through a 50 µm mesh. One day prior to the start of the experiment (i.e. on 21 May), zooplankton communities were collected at four sites in bays and along the shore in the unheated area, partly overlapping with the site for roe strand collection in the unheated area. Plankton nets of 20 and 70 µm mesh size were lowered to the target depth (<1 m), pulled horizontally for approximately 5 min at an average speed of 1 knot (0.5 m/s), and thereafter quickly retrieved to the surface to empty the collected plankton. Active sampling was conducted for a total of 3 h. Collected plankton were kept in an approximately 700 l tank filled with filtered water from the unheated area until being added to the mesocosms. On 21 May, 7.5 l of this well-mixed plankton mixture was added to each mesocosm.

The experimental temperature in each mesocosm was manipulated by either heating or cooling. Heating experimental conditions were achieved using turned-on thermostats (Eheim 300 W) placed in the centre of the mesocosm, and cooling conditions were realised by surrounding the mesocosms with a flow-through system of water from the unheated area (figures 1b and 2). For standardization, all mesocosms had thermostats in them regardless of them being turned on or off. We deployed a temperature logger (HOBO UA-001-64 Pendant Temperature Data Logger) at 50 cm depth in each mesocosm and recorded water temperature every hour since the temperature manipulation started. After averaging temperatures over the duration of the experiment, a thermal gradient of 14–25°C was maintained among all 38 mesocosms and within each fish treatment (figure 3). On this continuous gradient, temperatures in mesocosms of each fish treatment were evenly distributed across the full gradient (figure 3). The temperature fluctuated within each mesocosm owing to exposure to natural air temperature variation (electronic supplementary material, figure 3). Each mesocosm was aerated on the bottom with approximately the same intensity of air flow using one air stone connected to an air pump (Airflow 400, IP44 230 V). The aeration saturated mesocosm water with oxygen (electronic supplementary material, Mesocosm water chemistry) and prevented temperature stratification.

(c) Sampling and sample processing

Zooplankton and chlorophyll *a* (chl *a*) were sampled prior to the addition of fish larvae to the mesocosms (on day 0 and day −1, respectively), in the middle of the experiment (day 9) and when approaching the end of the experiment (day 19) by sampling 3.3 l water from each mesocosm at 40 cm depth using a 0.66 l Ruttner water sampler. This way, zooplankton was sampled from the water column at 40 cm depth to 16 cm without removing too much water or too many zooplankton. The water was filtered

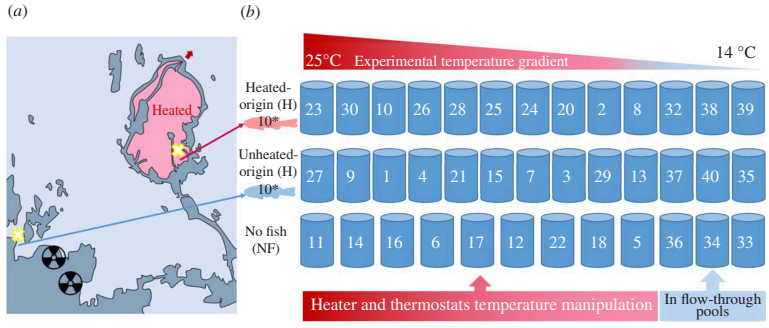

Figure 1. (*a*) Geographic layout of the study system: the heated area (H) and the unheated (UN) surroundings, previously separated also by a metal grid barrier at the outlet of the heated area (red arrow). The yellow crosses mark where in each area the roe strands were collected, from which larvae later were hatched to be used in the experiment. (*b*) Schematic of the experiment design testing the effect of the thermal origin of larvae (H versus UN), using 38 mesocosms (numbered 1–40, of which 19 and 31 did not exist): three rows for fish treatments (H, UN and no fish NF) and experimental temperature treatments resulting in a temperature gradient of 14–25°C achieved within each fish treatment, over the 21 day experiment period. For more details on the study system, mesocosm physical layout and temperature manipulation see electronic supplementary material, figure S1 and Experiment set-up.

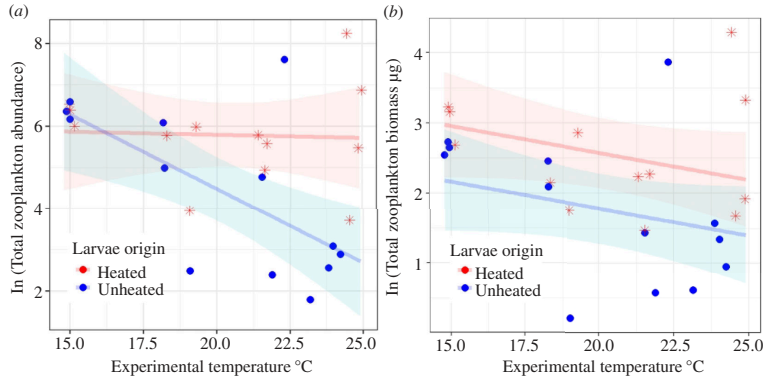

Figure 2. Zooplankton total abundance (*a*) and total biomass (*b*) at the end of the experiment in mesocosms with either fish larvae of heated population (H, red stars) or unheated population (UN, blue-filled points) along the experimental temperature gradient. The red and blue lines depict the predicted ln-transformed zooplankton abundance (*a*) or biomass (*b*) and the belts show their corresponding confidence intervals at 95%, using the best models (table 2, electronic supplementary material, table S7), which are ln(abundance)~origin × temperature for predicting abundance and ln(biomasss)~origin + temperature for biomass.

through a 70 µm mesh to keep most zooplankton on the mesh. We gently rinsed off each sample of the zooplankton collected on the mesh into a 100 ml brown bottle with tap water and added 4 ml Lugol's iodine solution into the same bottle. To estimate chl *a*, we filtered 500 ml water through a 47 mm glass fibre filter (Whatman™ GF/F) using a pressurized chamber. The GF/F filters were stored folded in aluminium foil and put in sealed bags at −20°C until they were processed for estimation of chl *a* concentration to approximate the phytoplankton biomass in our mesocosms [42]. Before adding perch larvae to the mesocosms, chl *a* and zooplankton were sampled and processed to confirm that there were no significant differences in phytoplankton and zooplankton communities among mesocosms of different treatments (electronic supplementary material, Mesocosm initial conditions). From 3 June, the remaining filtered water after sampling was returned to mesocosms to slow down the noticeable decrease in water level (mostly owing to evaporation under warm weather).

Zooplankton identification, counting and measurements were done using a stereo microscope (Leica M125C). We counted and measured individuals in 30 ml subsamples of the zooplankton mixture, and in the remaining volume, we continued counting only the taxa of which less than 50 individuals were counted in the first 30 ml. Copepoda were separated into nauplii, copepodite stages 1–3, stages 4–5 of order Calanoida (genus *Acartia* or *Eurytemora*), adults and order Cyclopoida. Cladocerans were separated into *Bosmina* sp., *Chydorus* sp. and *Podon* sp. Rotifers were identified to the genus *Keratella* (*K. cochlearis, K. quadrata* and *K. cruciformis*), *Brachionus and* class *Bdelloida*. We measured the individual body length to the nearest 0.01 mm of 20 or more individuals in each taxon for each mesocosm. Zooplankton abundance was the real count if the total volume of the sample was counted or extrapolated from a count of the first 30 ml in relationship to the sample's total volume. Zooplankton biomass was estimated from length measurements using body length–biomass relationships (electronic supplementary material, table S3) based on the abundance. Species richness was defined as the total number of present and different taxonomic groups.

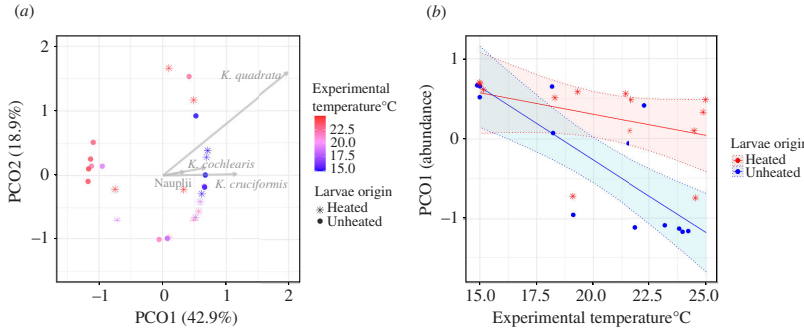

Figure 3. (*a*) Ordination of two main axes: PCO1 explaining 42.9% of the total variation, and PCO2, 18.9%, representing zooplankton community composition based on the abundance of different taxa in H mesocosms (star) and UN mesocosms (circles). Colour shows the mean experimental temperature in the mesocosm (from blue to red). Grey arrows show loadings of each taxonomic group on the PCO1 and PCO2 axes. The three species of *Keratella* and nauplii have the highest PCO1 scores. (*b*) Red and blue lines show that the difference in zooplankton composition (as given by PCO1) between larvae origins increased with temperature, according to PCO1 predicted by the best model in electronic supplementary material, table S7: *ln*(*PCO1*~*origin* × *temperature*). The opaque red and blue belts show the 95% confidence interval of the predicted PCO1. The red and blue points show PCO1 scores in H and UN mesocosms. For ordination and PCO1 prediction based on biomass of each taxonomic group, see electronic supplementary material, figure S6.

To estimate chl *a* concentrations, we processed the GF/F filters as follows: we cut each filter into half (exact proportion measured for accuracy) and put one of the halves directly into a 5 ml screw cap vial filled with 96% ethanol and then kept it in darkness at 4°C for 22.5 h for chl *a* extraction. All samples were shaken vigorously halfway through the extraction. Samples were thereafter centrifuged at 5000 r.p.m. for 5 min to sediment any particles. Three replicates of 200 µl of the supernatant of each sample were pipetted into three wells of a black solid-bottom 96-well plate. Fluorescence was measured at $\lambda_{ex/em}$ = 444 ± 12/675 ± 50 nm using a microplate reader (Hidex Sense) and converted to chl *a* concentration (µg l^{-1}) following the equation: chl *a* concentration (µg l^{-1}) = (RFU − 2766.5)/194.1 (electronic supplementary material, Mesocosm initial conditions). The samples and plates were kept cool in darkness and handled as fast as possible during the process.

Perch larvae used in the mesocosms were sampled at the end of the experiment, on experiment day 20 (11 June), by handmade drop nets of 1 mm mesh size that were slightly smaller than the mesocosm tank's diameter. We sank the drop net vertically to the mesocosm bottom, turned it horizontally at the bottom, pulled to the surface horizontally and picked up the fish from the net using tweezers. The fish were immediately put in a benzocaine solution (200 mg l^{-1}) to euthanize them and then transferred to containers with 80% ethanol for storage. If no fish were caught during five such fishing attempts, we deemed that no fish was left in the mesocosm and moved on to the next. In total, 138 fish larvae were caught. For the first three mesocosms, we repeated the same fishing process and emptied the tanks to validate the drop net method to see if there were missing fish to be caught. Despite the fact that no fish were caught during this control, we found 16 fish alive 22 days after the experiment ended (2 July) when we emptied all mesocosms. We deemed the sum of both occasions to be the best estimate of how many fish larvae survived the experiment (until 11 June). The number of fish captured on both occasions (11 June and 2 July) and the total can be found in electronic supplementary material, table S4.

Perch larvae hatched from the selected five roe strands of each origin were also sampled on 22 May after mesocosm larvae had been taken out from the hatching aquarium for inoculation, to measure their size (electronic supplementary material, tables S2, S5), as an approximation for the inoculated larvae. Thirty larvae were sampled from each aquarium using a hand net and stored in a container at −20°C with water. Body lengths of both mesocosm-caught (after experiment) and aquaria-caught (before experiment) perch larvae were thereafter measured to the nearest 0.01 mm using a stereo microscope. Fish wet weights were measured to the closest 0.1 mg after we patted them dry on both sides twice to minimize alcohol residual.

(d) Statistical analyses

All data processing and statistical analyses were conducted in R, v. 4.0.2, R Core Team 2014 [43]. Responses in the zooplankton community at the end of the experiment were our main focus. We assembled five different response variables: zooplankton abundance, biomass, species richness, composition by taxa and composition by size. Zooplankton abundance and biomass were ln-transformed prior to analyses. The zooplankton composition by taxa was represented by scores of the first ordination axis (PCO1) of an analysis of principal coordinates made on zooplankton taxa (based on zooplankton abundance or on biomass per each taxonomic group at the end of the experiment). It was achieved in two steps: first, we calculated a distance matrix of Bray–Curtis dissimilarity indices [44] using the function vegdist in package 'vegan' [45] on the raw data; then we used the function capscale to conduct an analysis of principal coordinates: capscale(matrix ~ 1). We grouped calanoid copepodite stages 4–5, adult *Eurytemora* and *Acartia* and cyclopoid copepods together as Copepoda because the numbers were low (owing to fish predation, see electronic supplementary material, Fish predation). We also recorded variance found between zooplankton taxa (species scores on PCO1) to investigate whether some taxa were particularly influential in driving the variation in zooplankton composition among mesocosms. The zooplankton composition by size was estimated by the proportion of large-size (>200 µm)

individuals. We found that 200 µm was a suitable threshold to separately group large- and small-sized zooplankton for their clear difference in abundance. It also separated the zooplankton species/taxonomic groups; copepods were larger than 200 µm and the three species of *Keratellas* were below 200 µm.

We used analysis of covariance (ANCOVA) to test whether the thermal origin of the larval fish (abbreviated as 'origin', as a categorical variable with discrete levels: H and UN), experimental temperature (as a continuous variable) and their interaction influenced these five zooplankton response variables at the end of the experiment. We then made predictions for abundance, biomass, scores of PCO1 and proportion of large individuals using the best model selected from established models using the three available variables (see table 2 for example model formulas). We identified the best model by ranking the established models based on the significance of pairwise likelihood ratio tests on sequential models of increasing complexity [46]. For zooplankton abundance, biomass and PCO1, we used linear regression in base R to establish models. For a proportion of large individuals, models were established using the R-package betareg [47].

We also tested the effect of thermal origin, experimental temperature and their interaction across the three sampling dates in the experiment on zooplankton abundance and biomass, using the model selection approach as above. The experimental temperature was calculated for each of these experiment days by averaging the hourly measurements from day 0 up until the experiment day in question. We also added experiment day to the models to account for the effect of time and the random effect of mesocosms. Models (electronic supplementary material, table S6) were established using the R-package lme4 [48].

Fish larvae survival, body length and weight at the end of the experiment were also analysed to evaluate their confounding effect on the thermal origin. Fish larvae weights were ln-transformed prior to analyses. Fish survival rate was calculated as the number of total larvae caught (electronic supplementary material, table S4) divided by 10, i.e. the initial number of fish larvae inoculated in each fish-present mesocosm. These values were then square root transformed to account for heteroscedasticity. Similarly, for zooplankton responses, we used ANCOVA to investigate whether there were fish-related variations between H and UN mesocosms.

Data visualization and processing were done using the packages within the tidyverse collection [49].

3. Results

Fish larvae thermal origin affected zooplankton total abundance, which also differed depending on experimental temperature (table 1, figure 2*a*). Zooplankton abundance at the end of the experiment was constant along the temperature gradient in H mesocosms, whereas it decreased with temperature in UN mesocosms (figure 2*a* and table 2). The effect of the thermal origin of the fish on zooplankton total abundance was evident throughout the experiment (electronic supplementary material, figure S4 and table S6).

Similarly, for zooplankton total biomass, there was an effect of fish thermal origin at the end of the experiment (table 1, figure 2*b*, electronic supplementary material, table S7), but across the three sampling dates, no model was significantly better than the null model (electronic supplementary material, figure S5 and table S6). Whereas zooplankton biomass decreased with temperature in both fish treatments, at the end of the experiment it was overall higher in H than UN mesocosms over the temperature gradient (figure 2*b*, electronic supplementary material, table S7).

While species richness did not differ significantly between H and UN mesocosms (ANCOVA, $F_{1,22} = 4.23$, $p = 0.096$), the fish origin affected zooplankton compositional variation as described by PCO1 (table 3; similarly for both when based on abundance or biomass). The effect of temperature on zooplankton composition also varied with fish origin (table 3). Scores on PCO1 based on both abundance and biomass remained nearly constant with temperature in H mesocosms but decreased with temperature in UN mesocosms (figure 3*b* and electronic supplementary material, figure S6b). The four taxa that partitioned the most variance on PCO1 (shown by the four longest arrows in figure 3*a* and electronic supplementary material, figure S6a), were three species of *Keratella*s and copepod nauplii. All four taxa had positive PCO1 scores, and as PCO1 decreased with experimental temperature in UN mesocosm, this suggests that these four taxa were negatively affected by larvae originated from the unheated area and the effect accentuated with temperature (figure 3*b* and electronic supplementary material, figure S6b). In contrast, the model of the PCO1 suggests that consumption by larvae from the heated population did not alter the zooplankton composition as temperature increased (figure 3*b* and electronic supplementary material, figure S6b), although the consumption of zooplankton total biomass did increase as temperature increased (figure 2*b*).

Similarly, there was also an interactive effect of the thermal origin of fish and experimental temperature on the proportion of large-sized zooplankton (figure 4, electronic supplementary material, table S7). The proportion decreased with temperature in H mesocosms but increased in UN mesocosms, rendering a smaller proportion of large-sized zooplankton in mesocosms with larvae originated from the heated than unheated populations at higher temperatures, especially in the eight warmest mesocosms (figure 4). We also found a significantly smaller proportion of copepods in those H than those in UN mesocosms (electronic supplementary material, figure S7). This suggests that the larvae of heated origin affected large zooplankton more than the larvae of unheated origin. This is in line with the result found in the zooplankton taxonomic composition response, where heated-origin larvae did not affect the four small-bodied taxa as much as unheated-origin larvae did.

Fish larvae had a clear top-down effect on zooplankton, shown by lower abundance and biomass of zooplankton (electronic supplementary material, figure S8 and Fish predation), and lower species richness (ANCOVA, $F_{2,32} = 33.94$, $p < 0.001$), in fish-present mesocosms compared to NF mesocosms (note that there was no difference in the initial conditions, see electronic supplementary material, Mesocosm initial conditions). Furthermore, the effect of fish thermal origin on zooplankton responses was probably not owing to a difference in fish abundance or growth as survival and the final length of fish larvae did not differ between H and UN mesocosms (ANCOVA on survival: $F_{1,22} = 0.44$, $p = 0.48$; length: $F_{1,134} = 2.58$, $p = 0.111$). However, individual H larvae slightly gained more weight than the ones of unheated origin during the experiment (ANCOVA, $p = 0.038$, $\eta^2 = 0.03$).

Table 1. Results of ANCOVA on the effects of larvae thermal origin (heated/unheated), temperature and the interaction between origin and temperature on zooplankton community abundance and biomass at the end of the experiment (day 20). The asterisk symbol indicates significant results ($p < 0.05$).

zooplankton	explanatory variables	$F_{1,38}$	*p*-value
total abundance	origin	5.84	0.024 *
	temperature	5.12	0.034 *
	origin × temperature	4.82	0.039 *
total biomass	origin	4.60	0.043 *
	temperature	2.33	0.141
	origin × temperature	1.30	0.266

Table 2. Result of model selection for finding the best model (marked in bold) to predict zooplankton abundance at the end of the experiment, as well as the coefficients of each included term of the best model. The established models are labelled as null, first, second and third with increasing complexity. Results of pairwise likelihood ratio tests are shown with asterisks representing the significance level. 'Temperature' coefficient −0.02 shows that zooplankton abundance remained nearly constant in H mesocosms and the coefficient −0.34 for the interaction 'origin × temperature' shows that zooplankton abundance decreased with temperature in UN mesocosms. Note that the coefficient for 'origin' 5.58 suggests that when the experimental temperature is 0°C, the abundance in UN mesocosm would be 5.58 units higher than the abundance in H mesocosms, which is of little biological meaning for our experimental temperature gradient, as the effect is taken over by the interaction term.

model selection				best model estimates	
response	model	formula	significance	terms	coefficients
ln(total zooplankton abundance + 1)	null	1			
	first	temperature	0.07	origin	5.58
	second	origin + temperature	0.02*	temperature	− 0.02
	third	origin × temperature	0.03*	origin × temperature	− 0.34

Table 3. Results of ANCOVA on zooplankton composition by taxa, represented by the scores of PCO1 generated from distance matries based on abundance or biomass per each zooplankton taxonomic group. The asterisk symbols indicate significant results (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Zooplankton composition given by PCO1 was affected by the thermal origin of fish larvae (heated/unheated), experimental temperature and their interaction.

distance matrix based on	variables	*F*	*p*-value
abundance	origin	16.65	<0.001 ***
	temperature	9.85	0.005 **
	origin × temperature	5.64	0.027 *
biomass	origin	19.69	<0.001 ***
	temperature	13.4	0.001 **
	origin × temperature	7.2	0.014 *

Larvae originating from different thermal populations thus have a different top-down effect on the zooplankton community, indicated by the observed differences in zooplankton abundance, biomass, species composition and size composition between H and UN mesocosms. The differences found in zooplankton responses between H and UN mesocosms did not, however, cause any differences in phytoplankton biomass between H and UN mesocosms, indicated by their similar chlorophyll *a* concentrations (electronic supplementary material, figure S9, ANCOVA, $p = 0.462$). Fish larvae did, however, have indirect top-down effects on phytoplankton, as chl *a* was higher in mesocosms with fish than in NF mesocosms, both throughout and at the end of the experiment (electronic supplementary material, figure S9). Taken together, this indicates that while fish larvae had top-down effects on zooplankton that in turn affected phytoplankton, the differences in these top-down effects depending on fish thermal origin did not cascade down to phytoplankton.

4. Discussion

We found that the thermal origin of fish larvae affected the abundance, biomass, as well as taxonomic and size composition of their zooplankton prey community differently, depending on the temperature in a mesocosm warming experiment. While controlling for initial prey community composition and other conditions among all mesocosms, no difference in fish abundance and body length at the end was found depending on the thermal origin, suggesting that the observed effect of fish thermal

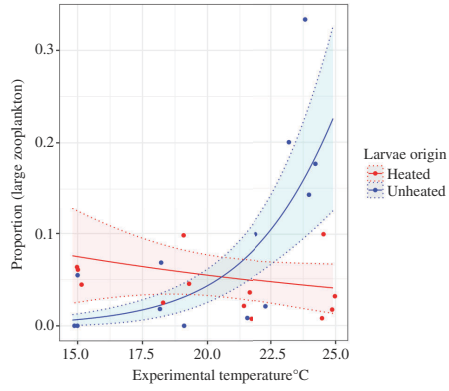

Figure 4. The proportion of large-sized zooplankton in H mesocosms (red points) and UN mesocosms (blue points) and predictions of it (and 95% confidence interval) made from the best model P(large zooplankton)~origin × temperature (electronic supplementary material, table S7) shown as the red and blue lines (belts for the confidence intervals). The proportion of large individuals is predicted to remain nearly constant at a low level in the H mesocosm and increase with temperature in the UN mesocosm.

origin on zooplankton was probably owing to differences in the feeding of perch larvae depending on their origin. This further suggests that perch may have responded to the long-term extensive heating in ways that resulted in a reduced top-down control on zooplankton.

The difference between zooplankton prey community responses to the presence of fish of heated and unheated origin might have arisen from a few non-exclusive mechanisms. First, perch from the chronically warmed environment may have physiologically adapted such that they require less food to sustain themselves under elevated temperatures, owing to a lowered metabolic rate [16,50]. Specifically for our study populations, adult perch from the heated area display significant metabolic thermal compensation and lowered heart rate at high temperatures, compared with adults from the unheated area [25]. Such warming-induced metabolic changes are commonly due to phenotypic plasticity [51], but evidence shows that evolutionary adaptations can also cause such metabolic changes, potentially selected to compensate for higher energy losses at high temperatures [16]. This potential lower food requirement of fish from the heated area might be why zooplankton abundance remained constant along the temperature gradient in mesocosms with larvae of heated origin, while the top-down effect of larvae of unheated origin increased with temperature, probably linked to increased feeding rates owing to increased (not-adapted) metabolism (figure 2*a*).

Second, larvae of heated origin may have reduced attack rates overall, which would directly decrease top-down effects on zooplankton. When exposed to high temperatures, some organisms display reduced attack rates even after acclimatization to warmer environments [6,50], although warming-induced evolution of fish attack rates has not yet been shown. It is also possible that perch of heated origin have adapted to feed on an already warm-adapted prey community in the heated area [17], and thus fed less efficiently when exposed to an unfamiliar prey community from the unheated area in the mesocosms, resulting in the observed lower top-down effect on zooplankton compared with the larvae of unheated origin (figure 2*b*). A follow-up experiment with a full factorial set-up manipulating the origins of both fish and zooplankton, in addition to the current experiment design, would help clarify this.

Third, larvae of heated origin may have evolved in ways that allow them to reach higher growth and development rates in high-temperature environments [26], potentially explaining the slight positive yet significant weight gain by the larvae of heated origin compared with the larvae of unheated origin. Young (though non-larval) perch of heated origin also have a faster growth rate [15] and earlier reproduction [52] in their heated home environment. Ultimately, these changes can lead to changes in body size [15,53] which determines the size of prey that larval fish are able to consume and/or prefer to feed on [54]. A larger gape size enabling an earlier shift to feed on larger-sized zooplankton [55] of larvae of heated origin could explain why we found proportionally less large-sized zooplankton and specifically fewer copepods in the presence of these larvae than of unheated-origin larvae at high temperatures (figure 4, electronic supplementary material, figure S7), and a larger negative effect of the larvae of unheated origin on the small-bodied zooplankton taxa (figure 3). This could also explain why the abundance did not decrease as much as biomass did in zooplankton communities in mesocosms with the larvae of heated origin as temperature increased (figure 2). It is also possible that the larvae of heated origin had a general shift to larger-sized zooplankton which did not change their relative prey consumption based on taxonomic groups (figure 3*b*, electronic supplementary material, figure S6b), but resulted in the increase in the total biomass with temperature (figure 2*b*).

We cannot rule out the contribution of maternal effects to the differences in fish top-down effects between the thermal origins, although we controlled for egg size of candidate roe strands (as indicated by roe strand width [55]) for the hatching of the perch larvae used in the experiment. Despite our selection effort, heated-origin roe strands were slightly wider than the unheated ones (electronic supplementary material, Roe strand collection and larvae selection). This may have resulted in the observed slightly larger body length in H larvae than in UN larvae at inoculation (electronic supplementary material,

table S5), despite hatching around the same time. This difference in body length could have contributed to potential prey selection differences and thus observed differences in prey composition. However, no difference was found in the initial weight of perch larvae (electronic supplementary material, table S5), suggesting that any potential maternal effects were probably small. Nevertheless, without more comprehensive testing for the genetic base of local adaptation relating to warming and genome-wide association studies integrating physiological and life-history traits, we cannot conclude which mechanism of the four discussed above is more likely to have caused the observed prey community responses to the presence of fish of different thermal origin.

Besides the direct top-down effect of fish larvae on zooplankton, we also investigated its indirect effects on the lowest trophic level in our mesocosms. We found that the differences in top-down effect between H and UN larvae on zooplankton probably did not cascade down to affect phytoplankton community biomass as suggested by the lack of a difference in chlorophyll *a* concentration between H and UN mesocosms (electronic supplementary material, figure S9). On the contrary, the general top-down effect from larvae independent of origin cascaded down to affect phytoplankton, as evident in a difference between chl *a* concentration between mesocosms with (H and UN) and without (NF) fish (electronic supplementary material, figure S9). The lack of a fish origin effect on phytoplankton biomass might be explained by the fact that primary producers are not susceptible to the moderate changes in the feeding of top predators as the intermediate trophic levels [13], i.e. zooplankton in our experiment. It could also be that other responses in the phytoplankton communities occurred than in the variable we measured (chl *a* concentration), e.g. in phytoplankton community species or size composition.

A few factors should be taken into consideration when interpreting our results. Our mesocosms were open to the atmosphere, resulting in their exposure to one storm event and to the potential colonization of other organisms. The storm that triggered a short power shutdown reduced the experimental temperature in all mesocosms to a similar level (electronic supplementary material, figure S3). This incident did not affect the overall temperature gradient among mesocosms over the experiment period nor did it remove the variation between them. Most importantly, the storm equally affected all mesocosms regardless of their fish treatment, so our main finding of the larval thermal origin effect on zooplankton should remain valid. Furthermore, the open atmosphere conditions may be more relevant to conditions in nature and thus provide more accurate predictions than studies conducted at constant temperatures [56]. We focused on zooplankton community responses and thus did not sample non-zooplankton prey organisms, e.g. chironomids, in the mesocosms. Those other invertebrates could have been part of the larval fish diet in addition to zooplankton or they could have also been predating on zooplankton communities besides the fish larvae [57]. However, as more than 99% of the fish larvae in the mesocosms were smaller than 20 mm, they were probably unable to efficiently consume larger prey such as chironomid larvae or pupae [58]. We found no difference in the presence of chironomids between H and UN mesocosms (electronic supplementary material, Other organisms). Thus, we believe the observed differences in zooplankton prey communities are driven by the thermal origin of fish larvae. Other factors that potentially confound the heated and unheated origin of our experimental larvae are environmental factors other than temperature that vary between the areas from which H and UN larvae originated. However, as the two areas were once a single area, environmental differences probably stem from the four-decade-long heating. We also expect any such variation (e.g. in primary production, vegetation biomass) to be outweighed by the unusually large temperature difference for natural systems (i.e. 5–10°C) between areas in terms of its effect on the ecosystem and the organisms therein. Therefore, the observed effects of larval origin on their zooplankton prey are most probably a consequence of long-term warming. Moreover, the long-term warming has occurred at the scale of a whole ecosystem, which makes findings in our specific study populations highly relevant in the context of global warming.

In conclusion, we show from direct measurements of zooplankton prey communities that wild predators' responses to multi-generational whole-ecosystem warming can induce variation in their top-down effects. The overall reduced feeding found in the larvae of heated origin was specifically evident in high-temperature environments, indicating that warming-induced changes in top-down effects may buffer potential negative effects of warming on the prey communities. Our experimental findings using a perch population heated for multiple generations aids the understanding of how warming may affect eco–evo feedback loops by showing that potential adaptations in predators to long-term warming can propagate to affect their prey community via feeding interactions. We, therefore, call for experiments generalizing our novel findings, to test whether responses to multi-generational warming in other fish and/or older life stages would cascade down to affect lower trophic levels and to further investigate the mechanisms by incorporating evolutionary and quantitative genetic methods—to infer whether these responses to warming are owing to local adaptation, maternal effects or others, in order to better predict community-wide impacts.

Ethics. This experiment was conducted in accordance with national regulations for animal care, and the experimental design and practices were reviewed and approved by the regional review board for ethical animal experiments in Uppsala, Sweden. Approved permit no.: Dnr 5.8.18-04546-2021, permit holder A.G.

Data accessibility. The data used in this manuscript can be accessed from the electronic supplementary material and Zenodo [59].

Supplementary material is available online [60].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. J.N.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; M.H.: conceptualization, data curation, investigation, methodology, supervision, writing—review and editing; A.G.: conceptualization, data curation, investigation, methodology, writing—review and editing; A.V.: supervision, writing—review and editing; A.G.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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Supplementary materials for research article

Multi-decadal warming alters predator's effect on prey community composition

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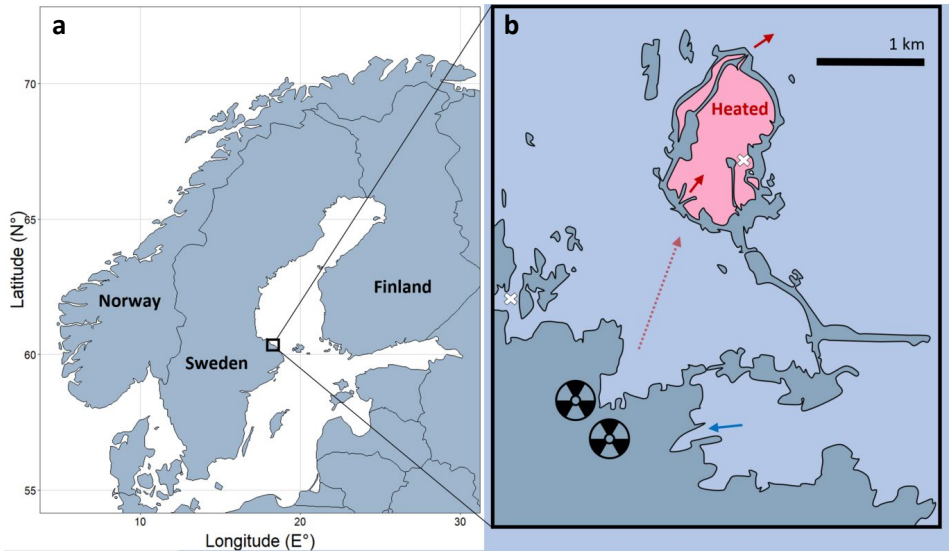


Figure S1. (a) Map of where the study system in figure 1a is located. The arrows in (b) show how the water is being circulated. The blue arrow marks the cooling water intake and direction that goes into the nuclear power plant. The red dashed arrow indicates the water flow through underwater pipes. Then the solid red arrows show where the heated water enters (bottom solid red arrow) and exits (upper solid red arrow) the heated area. (c) shows the more than 5 to 10 ° C temperature difference between the heated and unheated area. Daily mean water temperature measured in the heated vs. unheated area throughout the year are shown as red and blue opaque points. The thick red and blue lines mark the maximum temperature in the heated and unheated area. Water temperature was measured with temperature loggers during the ice-free period (March-December; 1989-2003; only including temperature measured at the same depth ~ 0.5 m and time for both areas).

The temperature difference between the heated and unheated areas (hereafter: H and UN area) from where the heated-origin and unheated-origin larvae (H and UN larvae) originated is unusually large (>5˚C, Figure S1c) for a natural system on an ecosystem warming scale and spans from 1980 to present. It can be compared to the temperature increase of 1.35 ˚C in the Baltic Sea due to climate change from 1982 to 2006 (Belkin, 2009).

Except for this large difference in water temperature, the two areas share many similarities. As they are adjacent, they share air temperature, weather conditions and any long-term environmental trends subjected to the whole region, such as climate change. The heated area was constructed by connecting existing small islands in the archipelago from the surrounding unheated area. They therefore also share the same general coastal habitat features such as shallow waters and near-shore. The two areas share similar water chemistry as the same water circulates within this 3 km radius area (Figure S1b). For a more specific comparison of key aspects between the two areas see Table S1.

Table S1. Environmental characteristics between the heated area (H) and unheated area (UN) where heated origin and unheated origin fish came from. We gathered the measurements taken where roe strands were collected in the two areas (Figure 1a).

Key aspect	Factor	H	UN	references
water chemistry	secchi-disc depth (m)	4-5	3-5	Sandström & Karås, 2002; Karås 1996a, Andersson et al. 1999
	turbidity (NTU)	2-3.5	3-5	
	salinity (PSU)	5	5	Snoeijs & Murasi, 2004
	dissolved nutrients (ug/L) in nitrate, silicate, phosphate	< 5% coefficient of variation		Hillebrand et al., 2010
physical characteristic	wave action	none	little to none	Snoeijs & Murasi, 2004
	turbulence (flow factor)	2	3	P. J. M. Snoeijs, 1989
exploitation	fishing (a common confounding factor exists in other warming studies)	none	none	Thoresson, 1992

Perch larvae thermal origin: study population

Evidence shows that the perch populations in H and UN areas might have separated into a heated and unheated population. In the heated area, perch have higher mortality (Lindmark et al., 2023). They grow faster when small (Huss et al., 2019; Lindmark et al., 2023). However, for large perch (at size > 10 cm and age > 2 years), the heating only has a positive effect on the body growth of females, but negative effects on male growth (van Dorst et al., 2023). Female perch of age 2-3 also have a smaller maturation size (Niu et al., 2023). The spawning season starts earlier for female perch in the heated area than for perch in the unheated area (Lukšienė et al., 2000; Niu et al., 2023). When experimentally exposed to acute warming, large perch from the heated area displayed lower metabolic rate, and tolerance to higher temperature (Sandblom et al., 2016). Perch from the heated area also have a higher resistance to parasites (Mateos-Gonzalez et al., 2015). These phenotypic trait divergences of perch in the heated area from perch in the unheated area support the separation of the two populations.

To the best of our knowledge, only two studies investigated the genetic differentiation between perch from the two areas. Using very limited numbers of microsatellite loci, allelic composition shifts in the major histocompatibility complex *(*MHC) alleles have been observed in the heated-area perch population (Björklund et al., 2015) and slight but significant genetic change that is consistent with the heated area perch isolation time (Demandt, 2010).

Genetic analysis

We sampled three individuals (larvae or egg) from each of these 30 roe strands (Table S2). Each individual was genotyped using 14 microsatellite loci (14_microsatellite_primer.xlsx in supplementary material) developed for perch and the genotypes can be found in microsatelliteDNA_genotypes.txt (electronic supplement). Fisher's exact probability test via

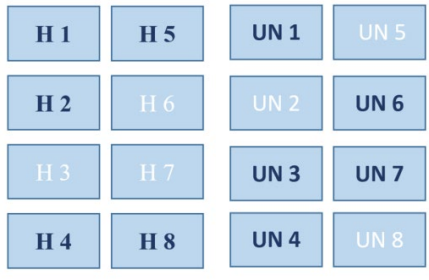
Genepop version 4.7.5 (https://genepop.curtin.edu.au/) options Population Differentiation and Fst & other correlations showed low but statistically significant genetic differentiation between the heated and unheated individuals (Fisher's exact probability test, $Chi^2 = 82.5$, $P < 0.001$; $Fst = 0.006$).

Result from this simple analysis is the only support prior to the experiment results that our sampled subpopulations (15 families = roe strands from heated and unheated population each) used in the experiment were somewhat separated. It was not feasible to follow the growth and measure comprehensive life-history trait differences of hatched larvae from each of these collected roe strands.

However, it should be noted that the significant genetic differentiation we found does not indicate any signal of adaption to warming. Instead it suggests a general allele frequency difference that can caused by drift or isolation or other warming-related secondary factors (e.g., change in parasitic load). All 14 loci are at non-coding regions and 14 loci is a small number to reveal signature of selection or potential adaptation to warming, not to mention relating to fish metabolism or feeding. The microsatellite analysis validated the **potential** of the warming response being evolutionary and brought more confidence when we talked about these potential pathways.

Roe strand collection and larvae selection

Table S2. Fifteen roe strands were collected from each of the heated and unheated area (location marked as in Figure 1) and provided for larvae used in H and UN mesocosms. For each population (from each area), eight roe strands with similar adhesion and width were selected and placed in separate 100 L aquariums, labelled as "H 1-8" and "UN 1-8". The other seven roe strands from each area were placed in 40 L aquaria, labelled as "Extra H 9-15" and "Extra UN 9-15". All aquaria were subjected to the same temperature and light regimes. Five roe strands were selected (H1, H2, H4, H5, H8 and UN1, UN3, UN4, UN6, UN7, marked as **bold in dark blue**) for each population from H1-8 and UN1-8. Roe strand selection for mesocosm larvae inoculation started primarily from the eight strands in the big glass aquaria as they had better hatching conditions compared to those in the small plastic aquaria. Hatching status of roe strands H1-8 and UN1-8 were recorded in the supplementary file: Record_hatchingstatus.xlsx.

	Heated population **15 roe strands**	**Unheated population** **15 roe strands**	**Selection of roe strands to inoculate larvae in fish-present mesocosm**	**Sampled for**	
				length and weight measurement	**DNA for microsatellite analysis**
Eight roe strands (1-8) in big glass aquarium (100L, better condition, primary resource for mesocosm larvae selection)			Two individuals from each of the five selected roe strand (marked bold in dark blue) from each population	30 individuals from each of the five selected roe strand from each population	Three individuals (larvae or egg) from all eight roe strands from each population
Seven roe strands (9-15) in small plastic aquarium (40L)			none	none	Three individuals (larvae or egg) from all seven roe strands from each population

Note that, despite our effort to select for as similar roe strands as possible to control for initial differences between mesocosm larvae, the five selected roe strands from the heated area were slightly larger (5.8 ± 1.2 cm) than those selected five from the unheated area (3.9 ± 0.4 cm; Welch Two Sample t-test $p < 0.05$, effect size 0.6).

These roe strands were upon collection likely maximum 10-day post spawning, based on water temperature measurements and the absence of visible embryos at the time of collection (Wang & Eckmann, 1994). There was no difference in temperature between hatching aquaria (Welch Two Sample t-test, $P = 0.94$, effect size in Cohen's $d = 0.014$) and the roe strands started hatching around the same time irrespective of origin. The first roe strand from the heated area started hatching on May 17th, all roe strands had started hatching on May 18th and more than half of each roe strand had hatched on May 21st. The first eggs from the unheated area hatched on May 18th, all had started to hatch on May 19th and more than half of each strand had hatched on May 21st (supplementary file: Record_hatchingstatus.xlsx).

Experimental set-up

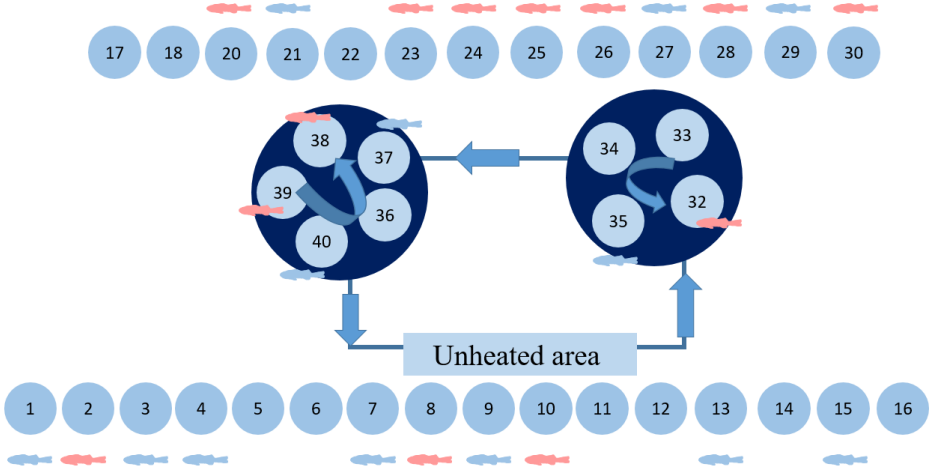


Figure S2. Schematic of experimental temperature manipulation in the 9 tanks that were "in flow-through pools" and the physical layout on the experiment site (Figure 1b). The red and blue fish symbol depict the fish treatment in H and UN mesocosms. These 9 tanks were placed in two bigger pools. The pools had water flow-through from and released back to the unheated area. These pools acted as a cooling system for these 9 tanks to reach a lower temperature (approximate to the real-time temperature of the unheated area) than it would have been without the cooling.

Temperature was manipulated either by turned-on thermostats heating or by cooling as illustrated in figure S2. The turned-on thermostats in the 29 heated tanks (Figure 1b) were set at 18, 22 and 26 °C. The three temperatures were decided so that together with the cooling treatment, temperature in the tanks would cover the water temperature range from that of the unheated area to the heated area. However, we ended up with a gradient of 14 - 25 °C in our mesocosms, shown in Figure S3. This is likely because 1) our mesocosms were outdoors and open to the atmosphere, so they fluctuated with the day and night temperature difference and they were exposed to extreme weather, e.g. storm, and to seasonal (spring to summer) air temperature increase; 2) placement of the mesocosms – some might have received more sunlight or more exposed to wind, and 3) variation in thermostats heating between mesocosms. Despite our calibration effort before the experiment started, we had to manually adjust the temperature of some thermostats during the experiment when e.g. the tank's water temperature has reached to the set temperature but the heater was still heating.

As shown in figure S3, temperature overlapped greatly between fish treatments throughout the experiment. Within each fish treatment, the temperatures of mesocosms were quite evenly distributed across the full gradient of 14 - 25 °C. In addition, the variance between each mesocosm was mostly consistent, i.e. despite the fluctuations a warmer mesocosm was always warmer. However, a heavy rainstorm swept our experiment site on experiment day 4-5, which led to a temporary power failure that shut down some thermostats and air pumps. This resulted in a sudden drop in temperature and between-mesocosm variance for a short period

(Figure S3). Due to all the above reasons, we retrospectively treated temperature as a continuous variable instead of the planned categorical factor with four levels.

The reason that tanks 19 and 31 did not exist (Figure 1b) was due to them leaking uncontrollably before the experiment started. Because of this, we had to change the initial experiment design of 40 mesocosms: 4 replicates * 4 experimental temperatures in both H and UN mesocosms (32 mesocosms) and 2 replicates * 4 temperatures in NF mesocosms (8 mesocosms). With 38 mesocosms, we changed to experiment design to 3 replicates * 4 temperatures in all H, UN and NF mesocosms (36 mesocosms), and assigned two additional mesocosms to the treatment with the highest temperature with fish. This was chosen as we expected most variation at the highest temperature.

Due to this achieved experimental temperature gradient and change of the initial experiment design, we ended up with the experiment set-up presented in the main text.

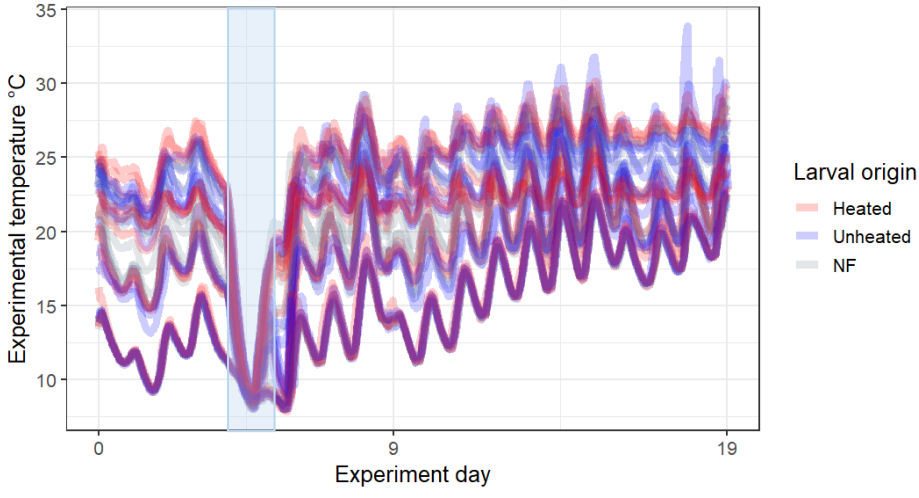


Figure S1. Consecutively hourly measured experimental temperature in every mesocosm shown by each line. The lines are coloured in red, blue and grey, depicting H, UN and NF mesocosms respectively. The temperature fluctuated rhythmically with diurnal cycles and increased slowly due to seasonal warming. The sudden drop in temperature and disappearance of between-mesocosm variance caused by the storm is marked by the opaque light blue box. As temperatures of all H, UN and NF mesocosms overlapped greatly and within each fish treatment, temperatures of mesocosms were quite evenly distributed across the full temperature gradient, the achieved temperatures among mesocosms were closer to a continuous variable.

Mesocosm initial conditions

We assessed the initial conditions among the mesocosms by phytoplankton community biomass and zooplankton community abundance to control for potential initial differences between mesocosms.

Phytoplankton community biomass was approximated by estimation of chl *a* concentration (µg/L) in our mesocosms (Huot et al., 2007). Chlorophyll *a* concentration in each mesocosm was derived from a chl *a* calibration line: Chl *a* concentration (µg/L) = (RFU - 2766.5)/194.1.

This equation was obtained from measured fluorescence of solutions with a gradient of chl *a* concentrations. We measured fluorescence (RFU) using a Hidex Sense microplate reader of 18 different solutions of a range of chl *a* concentrations (0-500 µg/L) diluted from standard stock solution (10mg/L).

There were no significant differences in chlorophyll *a* concentration between mesocosms of different fish treatments, which were sampled on experiment day -1 (ANOVA, chl *a* $F(2,35) = 0.859$, $P = 0.432$), indicating similar initial phytoplankton condition among H, UN or NF mesocosms.

There were no significant differences found in zooplankton abundance (sampled on day 0) among mesocosms of three fish treatments (ANOVA, zooplankton abundance $F(2,32) = 0.451$, $P = 0.641$).

This supports that the observed zooplankton responses were not likely due to the initial conditions of these aspects.

Table S3. Assumed length-to-weight relationships for zooplankton, where W is dry weight in µg, and L is body length in mm (prosome length for copepods, and the longest body axis excluding spines for all other taxa). All relationships are based on Bottrell et al. 1976.

Taxon	Conversion formula
Copepoda (incl. Nauplius)	ln W = 1.9526 + 2.399 * ln L
Keratella quadrata, K. cruciformis	ln W = ln(92.224) + 2.955 * ln L
Keratella cochlearis	ln W = ln(28.985) + 2.955 * ln L
Bdelloida	ln W = ln(16.949) + 3.0089 * ln L
Podon, Chydorus	ln W = 1.7512 + 2.653 * ln L
Bosmina	ln W = 3.0896 + 3.0395 * ln L

Initial and post experiment larvae

At hatching, larvae of the heated origin were larger on average (5.72 ± 0.42 mm) than the larvae of unheated origin (5.61 ± 0.39 mm; Welch Two Sample t-test $p < 0.001$, effect size glass's delta 0.5). This might be partly explained by the slightly larger roe strands from the heated area. Larval weight at hatching, however, showed no difference between larvae thermal origins (Table S5, $p = 0.609$, glass's delta 0.5). This also suggests for little difference in the fish larvae aspect between the mesocosms.

Table S4. Fish treatment for each mesocosm, and the number of larval fish caught on June 10th (experiment day 20) and July 2nd (22 days post the end of experiment) and the sum of both numbers under column "Total". No difference was found in the total number of fish caught between H and UN mesocosm, ANCOVA on survival: $F(1,22) = 0.44$, $P = 0.48$.

Mesocosm	Fish treatment	Number of fish caught		
		June 10th	July 2nd	Total
1	Unheated	2	0	2
2	Heated	8	0	8
3	Unheated	9	0	9
4	Unheated	9	1	10
5	NF	/	/	/

6	NF	/	/	/
7	Unheated	6	0	6
8	Heated	9	0	9
9	Unheated	1	0	1
10	Heated	2	1	3
11	NF	/	/	/
12	NF	/	/	/
13	Unheated	6	3	9
14	NF	/	/	/
15	Unheated	3	1	4
16	NF	/	/	/
17	NF	/	/	/
18	NF	/	/	/
20	Heated	9	1	10
21	Unheated	5	2	7
22	NF	/	/	/
23	Heated	0	0	0
24	Heated	5	1	6
25	Heated	6	0	6
26	Heated	2	1	3
27	Unheated	0	2	2
28	Heated	8	0	8
29	Unheated	9	1	10
30	Heated	2	1	3
32	Heated	7	1	8
33	NF	/	/	/
34	NF	/	/	/
35	Unheated	10	0	10
36	NF	/	/	/
37	Unheated	8	0	8
38	Heated	1	0	1
39	Heated	7	0	7
40	Unheated	4	0	4

Table S5. Weight and total length of perch larvae (non-inoculated larvae) from selected roe strands measured at the start of the experiment (Day 0) as a reference for larvae that inoculated into the mesocosms. Wet weight was measured in groups of five to increase precision. "/" marks individuals that could not be measured due to freeze damage. Roe strand number corresponds to the ones in Table S2. The average total length was calculated from supplementary file aquaria_larvae.xlsx.

Selected larvae roe strand	Weight of five individuals (g)	Average weight (g)	Average total length (mm)
H1	0.0024	0.00048	5.86
H2	0.0021	0.00042	5.56
H4	0.0018	0.00036	5.38
H5	0.0021	0.00042	/
H8	0.0023	0.00046	5.75

UN1	/	/	5.85
UN3	/	/	5.66
UN4	0.0022	0.00044	5.71
UN6	0.0021	0.00042	4.38
UN7	0.0019	0.00038	/

In the attached mesocosm_larvae.xlsx, we show wet weight, total length and length increment ($L_{day20} - L_{inoculation}$) of perch larvae that once inoculated in the mesocosms caught on June 10th (experiment day 20) and July 2nd (22 days post the end of experiment). Length increments are not included in any analysis for larvae caught on July 2nd as the values are not comparable with larvae caught on day 20. We did not calculate weight increment because wet weight at inoculation was so low (two orders of magnitude lower) that it is negligible compared to W_{day20}. On average, at the start of the experiment, a larva of heated origin weighed 0.428 ± 0.005 mg and a larva of unheated origin weighed 0.413 ± 0.003 mg (approximated from aquaria caught larvae of the same selected roe strands) compared to 11.388 ± 15.474 mg and 13.056 ± 12.183 mg, respectively, at the end of the experiment. Larvae from the heated origin grew to be heavier than those of unheated origin, ANCOVA, $F(1, 134) = 55.54$, $P < 0.0001$, $\eta 2 = 0.28$ but no difference found in larvae body length: $F(1,134) = 2.58$, $P = 0.111$).

Model selection

Table S6. Results of model selection and coefficients of significant terms from the selected best model (marked in bold) for zooplankton abundance and biomass throughout the experiment (three sampling days). The process was conducted by the same approach as described in Table 2. The sign * indicates significance level $p<0.05$. No best model was selected for biomass, so we do not report any model coefficients.

Model selection				**Estimates of the best model**	
response	model	formula	significance	term	coefficient
ln(total zooplankton abundance + 1) ~	null	day + random(day|mesocosm)		origin	-0.47
	first	temperature + day + random(day|mesocosm)	0.07		
	second	**origin + temperature + day +random(day|mesocosm)**	**0.02 ***	temperature	-0.059
	third	origin * temperature + day +random(day|mesocosm)	0.14		
ln(total zooplankton biomass + 1) ~	null	day + random(day|mesocosm)		No best model selected	
	first	temperature + day + random(day|mesocosm)	0.078		
	second	origin + temperature + day +random(day|mesocosm)	0.076		
	third	origin × temperature + day +random(day|mesocosm)	0.519		

Table S7. Results of model selection and coefficients of significant terms from the selected best model (marked in bold) for zooplankton biomass, PCO1 and proportion of large-size zooplankton at the end of the day conducted by the same approach as described in Table 2. The star symbol indicates significance level $p < 0.05$.

Model selection				Best model estimates	
response	model	formula	significance	terms	coefficient
ln(total zooplankton biomass + 1) ~	null	1		origin	-0.79
	first	temperature	0.067*	temperature	-0.077
	second	**origin + temperature**	**0.028***		
	thrid	origin × temperature	0.25		
PCO1~	null	1		origin	2.02
	first	temperature	0.0033*	temperature	-0.054
	second	origin + temperature	0.0088*	origin x temperature	-0.13
	third	**origin × temperature**	**0.027***		
P(large zooplankton) ~	null	1		origin	-9.44
	first	temperature	0.07	temperature	-0.07
	second	origin + temperature	0.83	origin × temperature	0.46
	third	**origin × temperature**	**< 0.001***		

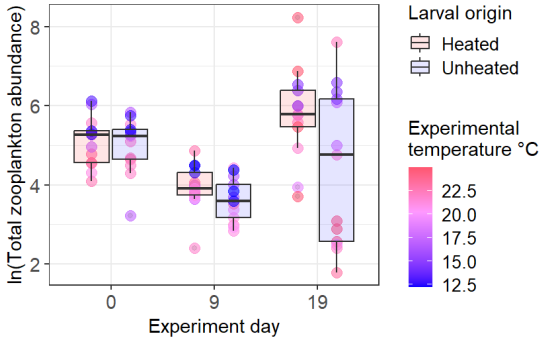


Figure S4. Overall zooplankton total abundance in H (red boxes) and UN (blue boxes) mesocosms on the three sampling occasions: experiment day 0, 9 and 19. The lower abundance in UN mesocosms appeared since the second sampling occasion. The filled-points show zooplankton abundance of each individual H or UN mesocosm. Their mean temperature (from the experiment start to that sampling day) is depicted by the colour of the point.

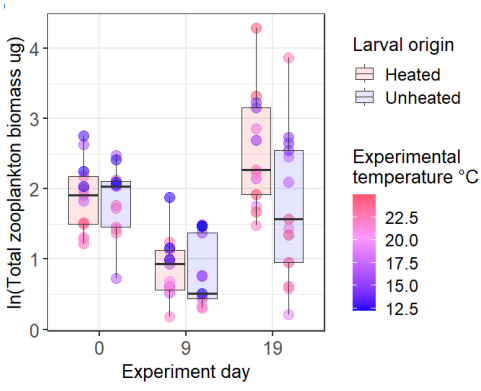


Figure S5. Overall zooplankton total biomass in H and UN mesocosms on the three sampling occasions: experiment day 0, 9 and 19, respectively shown by the red and blue boxes. The filled-points show zooplankton biomass of each individual H or UN mesocosm. The biomass in UN mesocosms was only lower on the third sampling day. Their mean temperature (from the experiment start to that sampling day) is depicted by the colour of the point.

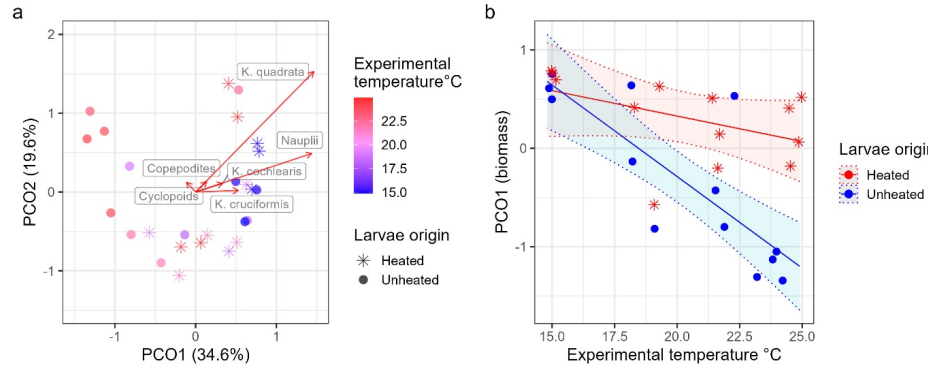


Figure S6. (a) Ordination showing scores of PCO1 (explaining 34.6% of the total variation) and PCO2 (explaining 19.6%) of each mesocosm based on biomass of different zooplankton taxa, on experiment day 19. H and UN mesocosms are shown as stars or circles. Colour shows their mean experimental temperature on the temperature gradient from cold (blue) to warm (red). The red arrows show the six zooplankton taxa having the highest absolute scores on PCO1. The six most influential taxa are the same as for zooplankton abundance. (b) Predicted PCO1 scores for H and UN mesocosms along the experimental temperature gradient, shown by solid lines in red and blue (95% confidence intervals shown by the dashed lines).

Zooplankton community species richness

Overall in mesocosms without fish, the number of taxonomic groups of zooplankton increased with time. At the end of the experiment (day 19), species richness was lower in mesocosms with fish than those without (ANCOVA, $F(2,32) = 33.94$, $P < 0.001$). On average, mesocosms without fish (NF) had 8.3 taxa, H mesocms had 5.8, and UN mesocosms had 4.9 and the differences come from copepodite stages 1-3, *Eurytemora* stages 4-5 and adult *Eurytemora*.

No difference was found in zooplankton species richness between H and UN mesocosms and no interactive effect was found between origin and experimental temperature. This supports that perch larvae primarily fed on copepods in our experiment (Figure S7).

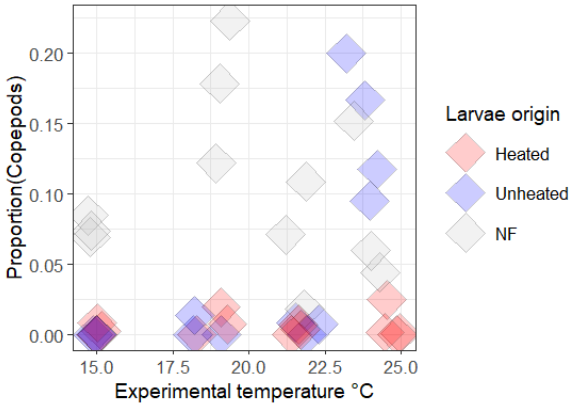


Figure S7. The proportion of copepods in H, UN and NF mesocosms (in red, blue and grey, respectively) across temperature. At the highest temperatures, the difference in the proportion of copepods between H and UN is large (origin effect in the eight highest temperature mesocosms: ANOVA, $F(1, 12) = 5.78$, $P = 0.033$), with proportionally more copepods in UN mesocosms than in H mesocosms

Mesocosm water chemistry

We measured dissolved oxygen (DO), salinity and pH using a portable multi-parameter Aquaprobe (AP 2000, AquaReadLtd, UK, see Aquaread.xlsx). DO measurement was calibrated with a 2-points method: 0% with zero oxygen tablets (Mettler Toledo) and 100% using wet paper towel surrounding the probe (following AquaReadLtd's manual instruction). The pH measurement was calibrated with a 3-points method using pH buffers at 7, 4 and 10.

Oxygen was saturated across the temperature gradient ($\geq$ 100%). DO concentration ranged from 8.32 to 9.40 mg/L. It decreased with temperature (ANCOVA, $F(1, 32) = 355.92$, $P < 0.0001$. Salinity range was 4.55 - 5.50 PSU across mesocosms. Also salinity increased with temperature, ($F(1,32) = 127.12$, $P < 0.0001$). Note that the change per degree temperature was low for both DO and salinity. Water pH's range was 7.89 – 8.10.

Most importantly, DO, salinity and pH did not differ between H, UN and NF mesocosms (ANCOVA, $F(2,32) = 1.524$, $P = 0.233$; $F(2,32) = 0.602$, $P = 0.554$; $F(2,32) = 3.031$, $P = 0.0623$), indicating that factors in water chemistry between mesocosms likely did not affect the observed zooplankton responses.

Fish predation

In support of the conclusion that observed differences in zooplankton communities were due to larvae thermal origin (rather than non-larvae aspects), we found that zooplankton abundance was higher in mesocosms without fish than in mesocosms with fish (ANCOVA, $P < 0.05$), independent of experimental temperatures (ANCOVA, $P = 0.93$; Figure S8). The relative abundance of nauplii and copepods was higher without fish (Figure S10 and Figure S7).

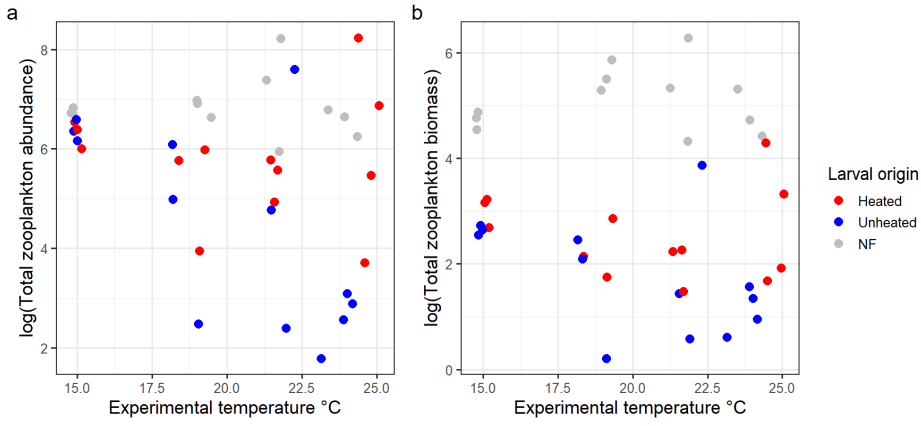


Figure S8. Scatterplot showing (a) zooplankton abundance and (b) biomass at the end of the experiment among H, UN and NF mesocosms in red, blue and grey filled-points.

Chlorophyll *a* biomass

No difference was found in chl *a* concentration between H and UN mesocosms (ANCOVA, $F(1, 72) = 1.10$, $P = 0.298$). Concentration of chl *a* was higher in mesocosms with than without fish (Figure S9, ANCOVA throughout experiment: $F(1,108)=3.53$, $P = 0.0628$; day 19: $F(1, 34) = 7.81$, $P = 0.0085$). Together with the higher zooplankton abundance and biomass found in NF mesocosms, this shows that fish top-down effects on zooplankton cascaded down to phytoplankton. Concentrations of chl *a* increased with temperature throughout the experiment regardless of fish presence (ANCOVA $F(1, 108) = 44.03$, $P < 0.0001$), or thermal origin ($F(1, 32) = 34.33$, $P < 0.0001$).

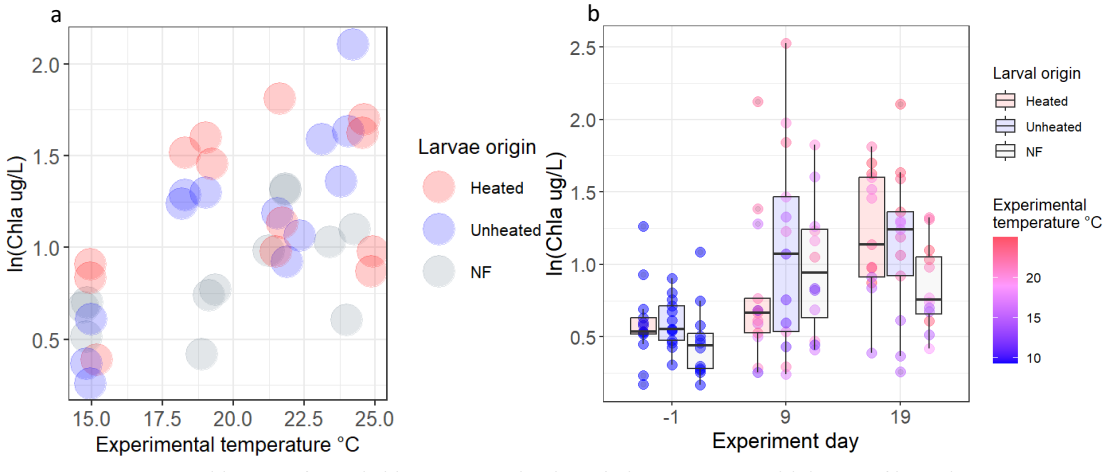


Figure S9. Natural log transformed chl *a* concentration in µg/L in mesocosms with larvae of heated (red) or unheated (blue) origin and mesocosms without fish (grey) across temperature (a) or sampling day (b) throughout the experiment. In (b) colours of circles indicate the experimental temperature, from cold (blue) to warm (red), of each mesocosm.

Other organisms

Our mesocosms were open to the atmosphere and thus midges, dragonfly and other organisms could have colonized the mesocosms. We did not systematically quantify the presence of other non-zooplankton organisms, as zooplankton communities are the primary prey of fish larvae, and we therefore designed our sampling and measurements focusing on only zooplankton. Still, we detected and noted presence of other potential zooplankton predator or prey for fish, such as chironomids larvae, zooplankton eggs at very low densities, polychaetes and nematodes.

We tested for presence of chironomid as a response at the end of the experiment. We found no difference in the presence/absence of chironomid between H and UN mesocosms (logistic regression for binomial response, $P = 1$), but NF mesocosms had higher presence of chironomids (positive coefficient from logistic regression, $P = 0.048$). This supports that the difference found in zooplankton communities between fish origin is not likely due to presence of chironomids. Similarly, zooplankton eggs and protists not accounted for are likely also of minor importance given larval perch feeding preferences (Mikheev & Wanzenböck, 2010).

Zooplankton composition

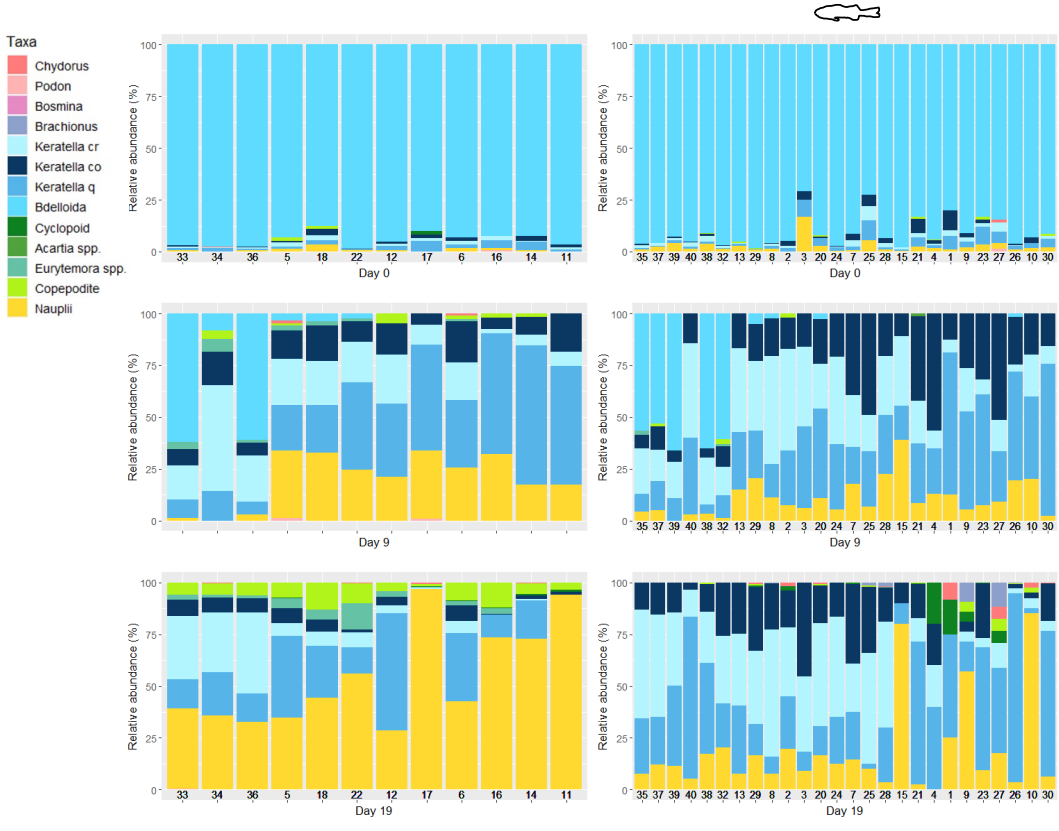


Figure S10. Zooplankton relative abundance per taxon is shown as a percentage on experimental days 0 (top), 9 (middle) and 19 (bottom) in NF mesocosms (left) and fish-present mesocosms (right). The numbered columns represent each mesocosm ordered from low to high temperature (left to right). Zooplankton community composition changed over time depending on whether there were fish or not in the mesocosm. It also changed with temperature in both mesocosms with and without fish. The initially dominant taxon, order *Bdelloida*, was replaced by *Keratella* spp. and copepod nauplii in all mesocosms over time. At the end of the experiment, copepod nauplii were dominant in some of the warmer mesocosms and more individuals of older stage copepodites, adult copepods and cladoceran emerged. Without fish, there were significantly more nauplii, copepodites and copopods at the end of the experiment.

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14_microsatellite_primer.xlsx: Primer information of used 14 tetranucleotide microsatellite loci (R. Gross, unpublished).

Locus Name	Repeat Motif	Fluorescence label	Forward Primer Sequence (5-3´)	Reverse Primer Sequence (5-3´)
Pflu4_4	ACTC	AT550	TCCACATCCTCTACTCTCCCA	TTCTGCCACTCATTGAGGC
Pflu4_22	AGAT	AT550	CATGGCTCCAATGACATCAG	CGCCTCTTTCCCTCTATTTG
Pflu4_40	AGAT	AT550	CATTGTCGCACACTTCTGCT	TTGAGTGCTCGGTACTTCCC
Pflu4_5	AAGG	AT565	TTGACATAGCGGTCAAGTCTGT	GATTTGGATGACTTGCGTAGG
Pflu4_20	AGAT	AT565	CACCGTACAGCAATCCCTAA	TGGGTAGTTGTCCCACATCA
Pflu4_44	AGAT	AT565	GAAAGGGTTTAAAGACGGCTG	TTATCCATTATGTCAGCGGC
Pflu4_1	AGAT	FAM	CCTGAAGGACAAGGAGAGGA	CATGTCCCAGTTTGGGATTC
Pflu4_10	ATCC	FAM	TCTGATTTCACAACGCAACA	CATGAAGTGTTCCCTCACCC
Pflu4_31	ACAT	FAM	GCTGACAAGCTAATGGCTCC	AACAGGACACTATGCCCACC
Pflu4_45	ATCC	FAM	TACCCTGACGCCTATGCTTT	TTGATTTGAGCTAGGTGATCCA
Pflu4_2	AATC	YYE	TACCGCCACCTGATTCAACT	CAGGACTGAAGCAATCAGCA
Pflu4_14	AGAT	YYE	TGTCGTTTGGTTAAGGCACA	GCTGATGCCGTTGAGTTTG
Pflu4_28	ACAG	YYE	TTGGGACAAACCTGGTAGATG	GGCAACACATCTTCATCCCT
Pflu4_42	ACAT	YYE	CGGACCAGGTTTCCTACAGA	TGACTCCATAACCCTCCACA

Record_hatchingstatus.xlsx: Roe strand width (cm) upon collection, egg development or larvae hatching status, and their aquaria water temperature (T °C) of the big aquarium roe strands (Table S2).
"Status" number #1 stands for that there was no visible eyes developed in the egg; #2: eyes of the some larvae became visible but no hatched larvae; #3: a few larvae hatched; #4: half or more of the eggs hatched; #5: all eggs hatched and #6: dead egg or larvae observed.
The roe strands from which we selected hatched larvae for use in the mesocosm experiment are noted as "x" in the "inoculated" row.
The 5 egg strands per origin were selected out of the candidates so that they had the most similar hatching status around the same time.

Date		**H 1**	**H 2**	**H 3**	**H 4**	**H 5**	**H 6**	**H 7**	**H 8**	**UN 1**	**UN 2**	**UN 3**	**UN 4**	**UN 5**	**UN 6**	**UN 7**	**UN 8**
	Width (cm)	6.9	4.9	5.9	4.1	6.4	3.7	4.1	6.7	4.2	3.9	3.6	3.6	3.6	3.6	4.3	3.5
10-May	T °C	8.2	8.3	8.2	8.2	7.7	7.6	7.5	7.5	8.2	8.1	8	8.1	7.9	7.6	7.5	7.8
	status	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12-May	T °C	10.3	10.3	10.3	10.4	9.8	9.7	9.7	9.8	10.3	10.3	10.3	10.3	9.9	9.8	9.8	9.8
	status	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13-May	T °C	14	13.9	13.8	14.1	13.1	12.8	12.7	12.8	14.3	14.2	13.8	14.4	13.2	13	12.6	12.9
	status	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14-May	T °C	14.1	14.2	13.8	13.8	13.5	13.3	13.1	13.2	14.3	14.4	13.8	13.9	12.8	13.7	13.1	13.4
	status	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16-May	T °C	13.4	13.6	13.2	13.4	13	12.9	12.6	12.8	13.8	13.9	13.3	13.8	12.9	13.2	12.6	12.8
	status	2	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1
17-May	T °C	14.5	14.5	14.3	14.4	13.9	13.8	13.6	13.7	14.2	14.8	14.4	14.8	13.9	14.1	13.8	13.9
	status	3	2	1	2	3	1	1	1	2	2	2	2	1	1	1	1
18-May	T °C	15	15.1	15.1	15.2	14.5	14.2	14.3	14.3	15.2	15.4	14.8	15.4	14.9	14.6	14.6	14.5
	status	3	3	3	3	3	1	2	3	3	3	3	3	2	2	2	1
19-May	T °C	15.8	16	15.6	15.7	15	14.9	14.6	14.8	16.1	16.2	15.6	16	15.1	15.2	14.6	14.9
	status	4	3	3	3	3	3	3	3	3	3	4	3	2	3	3	2
20-May	T °C	15.8	15.9	15.6	15.8	15.1	14.9	14.7	14.9	16.2	16.3	15.7	16.2	15.1	15.2	14.7	15
	status	4	4	2 & 6	4	4	3	3	3	3	3	4	4	2	3	3	3
22-May	inoculated	**x**	**x**		**x**	**x**			**x**	**x**		**x**	**x**		**x**	**x**	

Aquaread.xlsx: Measurements of dissolved oxygen (DO, in percent saturation and concentration), salinity (PSU), and pH by a portable multi-parameter Aquaprobe (Aquaread) of each mesocosm on experiment day 20.

Tank	Fish treatment	pH	DO (%)	DO (mg/L)	Salinity (PSU)
1	Unheated	7.97	111.5	8.32	5.25
2	Heated	7.98	111.5	8.8	4.77
3	Unheated	7.95	112.3	8.89	4.82
4	Unheated	7.93	112.3	8.68	5.22
5	nofish	7.97	108.8	8.81	4.76
6	nofish	7.99	110	8.85	5.03
7	Unheated	8.02	108.4	8.68	4.95
8	Heated	8.05	108	8.87	4.66
9	Unheated	8.06	109.3	8.72	5.17
10	Heated	8.1	109.7	8.45	5.24
11	nofish	8.07	108.3	8.39	5.19
12	nofish	8.01	109.5	8.72	4.94
13	Unheated	8.03	109.6	9.03	4.67
14	nofish	8.03	110.1	8.48	5.29
15	Unheated	8.06	110.5	8.77	4.99
16	nofish	8.01	111.2	8.62	5.35
17	nofish	8.02	109.5	8.53	4.85
18	nofish	8	110.4	8.94	4.69
20	Heated	8.06	109.5	8.88	4.8
21	Unheated	8.07	108.8	8.78	4.97
22	nofish	8.05	108.2	8.91	4.69
23	Heated	8.08	108.7	8.58	5.16
24	Heated	8.07	107.7	8.83	4.89
25	Heated	8.01	107.2	8.83	4.93
26	Heated	8.05	109.9	8.64	5.27
27	Unheated	7.98	109.2	8.52	5.19
28	Heated	7.99	109	8.83	4.93
29	Unheated	8.01	109.7	9.16	4.73
30	Heated	8.08	111	8.41	5.5
32	Heated	8.02	109.7	9.26	4.62
33	nofish	8	109.6	9.35	4.57
34	nofish	7.98	110.1	9.37	4.55
35	Unheated	7.96	110.4	9.34	4.57
36	nofish	8	108.9	9.27	4.58
37	Unheated	8.01	109.9	9.37	4.6
38	Heated	7.97	110.4	9.4	4.56
39	Heated	7.99	109.1	9.31	4.57
40	Unheated	7.89	109.4	9.33	4.61

aquaria_larvae.xlsx: Body length measurements of larvae hatched from the selected five roe strands of each heated and unheated origin. They were sampled the same day as the selected larvae inoculated in the mesocosms. The measurements were, therefore, used as proximations for the initial condition of the inoculated larvae in the mesocosms.

Roe strand ID	number	TL units	Roe strand width (cm)	Magnification	TL (mm)
Heated1	1	76	6.9	12.5	6.08
Heated1	2	78	6.9	12.5	6.24
Heated1	3	78	6.9	12.5	6.24
Heated1	4	75	6.9	12.5	6
Heated1	5	78	6.9	12.5	6.24
Heated1	6	77	6.9	12.5	6.16
Heated1	7	80	6.9	12.5	6.4
Heated1	8	79	6.9	12.5	6.32
Heated1	9	76	6.9	12.5	6.08
Heated1	10	79	6.9	12.5	6.32
Heated1	11	79	6.9	12.5	6.32
Heated1	12	75	6.9	12.5	6
Heated1	13	80	6.9	12.5	6.4
Heated1	14	77	6.9	12.5	6.16
Heated1	15	75	6.9	12.5	6
Heated1	16	75	6.9	12.5	6
Heated1	17	79	6.9	12.5	6.32
Heated1	18	81	6.9	12.5	6.48
Heated1	19	78	6.9	12.5	6.24
Heated1	20	72	6.9	12.5	5.76
Heated1	21	78	6.9	12.5	6.24
Heated1	22	75	6.9	12.5	6
Heated1	23	75	6.9	12.5	6
Heated1	24	70	6.9	12.5	5.6
Heated1	25	76	6.9	12.5	6.08
Heated1	26	77	6.9	12.5	6.16
Heated1	27	81	6.9	12.5	6.48
Heated1	28	79	6.9	12.5	6.32
Heated1	29	83	6.9	12.5	6.64
Heated1	30	79	6.9	12.5	6.32
Heated2	1	63	4.9	12.5	5.04
Heated2	2	73	4.9	12.5	5.84
Heated2	3	72	4.9	12.5	5.76
Heated2	4	67	4.9	12.5	5.36
Heated2	5	72	4.9	12.5	5.76
Heated2	6	70	4.9	12.5	5.6
Heated2	7	71	4.9	12.5	5.68
Heated2	8	74	4.9	12.5	5.92
Heated2	9	75	4.9	12.5	6
Heated2	10	71	4.9	12.5	5.68
Heated2	11	70	4.9	12.5	5.6
Heated2	12	72	4.9	12.5	5.76
Heated2	13	64	4.9	12.5	5.12
Heated2	14	68	4.9	12.5	5.44

Heated2	15	69	4.9	12.5	5.52
Heated2	16	72	4.9	12.5	5.76
Heated2	17	75	4.9	12.5	6
Heated2	18	71	4.9	12.5	5.68
Heated2	19	66	4.9	12.5	5.28
Heated2	20	66	4.9	12.5	5.28
Heated2	21	70	4.9	12.5	5.6
Heated2	22	70	4.9	12.5	5.6
Heated2	23	64	4.9	12.5	5.12
Heated2	24	75	4.9	12.5	6
Heated2	25	75	4.9	12.5	6
Heated2	26	68	4.9	12.5	5.44
Heated2	27	70	4.9	12.5	5.6
Heated2	28	62	4.9	12.5	4.96
Heated2	29	71	4.9	12.5	5.68
Heated2	30	64	4.9	12.5	5.12
Heated2	31	67	4.9	12.5	5.36
Heated2	32	67	4.9	12.5	5.36
Heated4	1	66	4.1	12.5	5.28
Heated4	2	70	4.1	12.5	5.6
Heated4	3	65	4.1	12.5	5.2
Heated4	4	67	4.1	12.5	5.36
Heated4	5	67	4.1	12.5	5.36
Heated4	6	68	4.1	12.5	5.44
Heated4	7	72	4.1	12.5	5.76
Heated4	8	65	4.1	12.5	5.2
Heated4	9	63	4.1	12.5	5.04
Heated4	10	65	4.1	12.5	5.2
Heated4	11	65	4.1	12.5	5.2
Heated4	12	64	4.1	12.5	5.12
Heated4	13	75	4.1	12.5	6
Heated4	14	65	4.1	12.5	5.2
Heated4	15	60	4.1	12.5	4.8
Heated4	16	63	4.1	12.5	5.04
Heated4	17	60	4.1	12.5	4.8
Heated4	18	67	4.1	12.5	5.36
Heated4	19	65	4.1	12.5	5.2
Heated4	20	66	4.1	12.5	5.28
Heated4	21	74	4.1	12.5	5.92
Heated4	22	71	4.1	12.5	5.68
Heated4	23	72	4.1	12.5	5.76
Heated4	24	66	4.1	12.5	5.28
Heated4	25	70	4.1	12.5	5.6
Heated4	26	69	4.1	12.5	5.52
Heated4	27	68	4.1	12.5	5.44
Heated4	28	65	4.1	12.5	5.2
Heated4	29	72	4.1	12.5	5.76
Heated4	30	68	4.1	12.5	5.44
Heated4	31	70	4.1	12.5	5.6
Heated8	1	77	6.7	12.5	6.16
Heated8	2	70	6.7	12.5	5.6
Heated8	3	75	6.7	12.5	6

Heated8	4	72	6.7	12.5	5.76
Heated8	5	71	6.7	12.5	5.68
Heated8	6	69	6.7	12.5	5.52
Heated8	7	74	6.7	12.5	5.92
Heated8	8	70	6.7	12.5	5.6
Heated8	9	75	6.7	12.5	6
Heated8	10	70	6.7	12.5	5.6
Heated8	11	70	6.7	12.5	5.6
Heated8	12	67	6.7	12.5	5.36
Heated8	13	72	6.7	12.5	5.76
Heated8	14	80	6.7	12.5	6.4
Heated8	15	65	6.7	12.5	5.2
Heated8	16	72	6.7	12.5	5.76
Heated8	17	79	6.7	12.5	6.32
Heated8	18	68	6.7	12.5	5.44
Heated8	19	74	6.7	12.5	5.92
Heated8	20	78	6.7	12.5	6.24
Heated8	21	73	6.7	12.5	5.84
Heated8	22	71	6.7	12.5	5.68
Heated8	23	67	6.7	12.5	5.36
Heated8	24	66	6.7	12.5	5.28
Heated8	25	67	6.7	12.5	5.36
Heated8	26	75	6.7	12.5	6
Heated8	27	70	6.7	12.5	5.6
Heated8	28	74	6.7	12.5	5.92
Heated8	29	60	6.7	12.5	4.8
Heated8	30	74	6.7	12.5	5.92
Heated8	31	80	6.7	12.5	6.4
Heated8	32	73	6.7	12.5	5.84
Heated8	33	76	6.7	12.5	6.08
UNheated1	1	74	4.2	12.5	5.92
UNheated1	2	69	4.2	12.5	5.52
UNheated1	3	76	4.2	12.5	6.08
UNheated1	4	72	4.2	12.5	5.76
UNheated1	5	67	4.2	12.5	5.36
UNheated1	6	69	4.2	12.5	5.52
UNheated1	7	75	4.2	12.5	6
UNheated1	8	75	4.2	12.5	6
UNheated1	9	75	4.2	12.5	6
UNheated1	10	75	4.2	12.5	6
UNheated1	11	73	4.2	12.5	5.84
UNheated1	12	72	4.2	12.5	5.76
UNheated1	13	74	4.2	12.5	5.92
UNheated1	14	76	4.2	12.5	6.08
UNheated1	15	70	4.2	12.5	5.6
UNheated1	16	71	4.2	12.5	5.68
UNheated1	17	70	4.2	12.5	5.6
UNheated1	18	72	4.2	12.5	5.76
UNheated1	19	76	4.2	12.5	6.08
UNheated1	20	72	4.2	12.5	5.76
UNheated1	21	73	4.2	12.5	5.84
UNheated1	22	73	4.2	12.5	5.84

UNheated1	23	74	4.2	12.5	5.92
UNheated1	24	74	4.2	12.5	5.92
UNheated1	25	71	4.2	12.5	5.68
UNheated1	26	72	4.2	12.5	5.76
UNheated1	27	71	4.2	12.5	5.68
UNheated1	28	75	4.2	12.5	6
UNheated1	29	77	4.2	12.5	6.16
UNheated1	30	72	4.2	12.5	5.76
UNheated1	31	80	4.2	12.5	6.4
UNheated3	1	74	3.6	12.5	5.92
UNheated3	2	76	3.6	12.5	6.08
UNheated3	3	75	3.6	12.5	6
UNheated3	4	75	3.6	12.5	6
UNheated3	5	71	3.6	12.5	5.68
UNheated3	6	75	3.6	12.5	6
UNheated3	7	64	3.6	12.5	5.12
UNheated3	8	71	3.6	12.5	5.68
UNheated3	9	75	3.6	12.5	6
UNheated3	10	66	3.6	12.5	5.28
UNheated3	11	74	3.6	12.5	5.92
UNheated3	12	68	3.6	12.5	5.44
UNheated3	13	72	3.6	12.5	5.76
UNheated3	14	73	3.6	12.5	5.84
UNheated3	15	67	3.6	12.5	5.36
UNheated3	16	71	3.6	12.5	5.68
UNheated3	17	73	3.6	12.5	5.84
UNheated3	18	77	3.6	12.5	6.16
UNheated3	19	71	3.6	12.5	5.68
UNheated3	20	60	3.6	12.5	4.8
UNheated3	21	80	3.6	12.5	6.4
UNheated3	22	73	3.6	12.5	5.84
UNheated3	23	67	3.6	12.5	5.36
UNheated3	24	72	3.6	12.5	5.76
UNheated3	25	68	3.6	12.5	5.44
UNheated3	26	65	3.6	12.5	5.2
UNheated3	27	66	3.6	12.5	5.28
UNheated3	28	67	3.6	12.5	5.36
UNheated3	29	65	3.6	12.5	5.2
UNheated3	30	67	3.6	12.5	5.36
UNheated3	31	74	3.6	12.5	5.92
UNheated4	1	68	3.6	12.5	5.44
UNheated4	2	70	3.6	12.5	5.6
UNheated4	3	72	3.6	12.5	5.76
UNheated4	4	70	3.6	12.5	5.6
UNheated4	5	76	3.6	12.5	6.08
UNheated4	6	70	3.6	12.5	5.6
UNheated4	7	78	3.6	12.5	6.24
UNheated4	8	71	3.6	12.5	5.68
UNheated4	9	66	3.6	12.5	5.28
UNheated4	10	74	3.6	12.5	5.92
UNheated4	11	74	3.6	12.5	5.92
UNheated4	12	72	3.6	12.5	5.76

UNheated4	13	70	3.6	12.5	5.6
UNheated4	14	65	3.6	12.5	5.2
UNheated4	15	69	3.6	12.5	5.52
UNheated4	16	76	3.6	12.5	6.08
UNheated4	17	72	3.6	12.5	5.76
UNheated4	18	76	3.6	12.5	6.08
UNheated4	19	73	3.6	12.5	5.84
UNheated4	20	74	3.6	12.5	5.92
UNheated4	21	65	3.6	12.5	5.2
UNheated4	22	72	3.6	12.5	5.76
UNheated4	23	75	3.6	12.5	6
UNheated4	24	74	3.6	12.5	5.92
UNheated4	25	74	3.6	12.5	5.92
UNheated4	26	67	3.6	12.5	5.36
UNheated4	27	70	3.6	12.5	5.6
UNheated4	28	74	3.6	12.5	5.92
UNheated4	29	77	3.6	12.5	6.16
UNheated4	30	70	3.6	12.5	5.6
UNheated4	31	65	3.6	12.5	5.2
UNheated4	32	67	3.6	12.5	5.36
UNheated4	33	70	3.6	12.5	5.6
UNheated6	1	55	3.6	12.5	4.4
UNheated6	2	65	3.6	12.5	5.2
UNheated6	3	67	3.6	12.5	5.36
UNheated6	4	50	3.6	12.5	4
UNheated6	5	55	3.6	12.5	4.4
UNheated6	6	52	3.6	12.5	4.16
UNheated6	7	54	3.6	12.5	4.32
UNheated6	8	55	3.6	12.5	4.4
UNheated6	9	58	3.6	12.5	4.64
UNheated6	10	60	3.6	12.5	4.8
UNheated6	11	50	3.6	12.5	4
UNheated6	12	52	3.6	12.5	4.16
UNheated6	13	54	3.6	12.5	4.32
UNheated6	14	40	3.6	12.5	3.2
UNheated6	15	61	3.6	12.5	4.88
UNheated6	16	60	3.6	12.5	4.8
UNheated6	17	45	3.6	12.5	3.6
UNheated6	18	60	3.6	12.5	4.8
UNheated6	19	50	3.6	12.5	4
UNheated6	20	55	3.6	12.5	4.4
UNheated6	21	57	3.6	12.5	4.56
UNheated6	22	65	3.6	12.5	5.2
UNheated6	23	51	3.6	12.5	4.08
UNheated6	24	65	3.6	12.5	5.2
UNheated6	25	54	3.6	12.5	4.32
UNheated6	26	50	3.6	12.5	4
UNheated6	27	45	3.6	12.5	3.6
UNheated6	28	60	3.6	12.5	4.8
UNheated6	29	48	3.6	12.5	3.84
UNheated6	30	48	3.6	12.5	3.84
UNheated7	1	66	4.3	12.5	5.28

UNheated7	2	55	4.3	12.5	4.4
UNheated7	3	77	4.3	12.5	6.16
UNheated7	4	65	4.3	12.5	5.2
UNheated7	5	74	4.3	12.5	5.92
UNheated7	6	65	4.3	12.5	5.2
UNheated7	7	69	4.3	12.5	5.52
UNheated7	8	70	4.3	12.5	5.6
UNheated7	9	71	4.3	12.5	5.68
UNheated7	10	67	4.3	12.5	5.36
UNheated7	11	60	4.3	12.5	4.8
UNheated7	12	73	4.3	12.5	5.84
UNheated7	13	64	4.3	12.5	5.12
UNheated7	14	65	4.3	12.5	5.2
UNheated7	15	63	4.3	12.5	5.04
UNheated7	16	60	4.3	12.5	4.8
UNheated7	17	63	4.3	12.5	5.04
UNheated7	18	70	4.3	12.5	5.6
UNheated7	19	65	4.3	12.5	5.2
UNheated7	20	67	4.3	12.5	5.36
UNheated7	21	63	4.3	12.5	5.04
UNheated7	22	65	4.3	12.5	5.2
UNheated7	23	66	4.3	12.5	5.28
UNheated7	24	64	4.3	12.5	5.12
UNheated7	25	61	4.3	12.5	4.88
UNheated7	26	62	4.3	12.5	4.96
UNheated7	27	59	4.3	12.5	4.72
UNheated7	28	60	4.3	12.5	4.8
UNheated7	29	63	4.3	12.5	5.04
UNheated7	30	65	4.3	12.5	5.2

mesocosm_larvae.xlsx: Weight and total length (TL) of perch larvae measured after capture from fish-present mesocosms on June 10th (Day 20) and July 2nd (22 days post the experiment end). "/" marks individuals that were caught on July 2nd and length increment was skipped as it is not comparable with larvae caught on experiment day 20.

Mesocosm	Date	Larval ID	Larval origin	Weight (g)	TL (mm)	Length increment (mm)
1	Experiment day 20	1	unheated	0.0048	10.79	5.18
1	Experiment day 20	2	unheated	0.0595	20.32	14.7
2	Experiment day 20	3	heated	0.0072	11.75	6.03
2	Experiment day 20	4	heated	0.0033	9.84	4.13
2	Experiment day 20	5	heated	0.0056	11.11	5.4
2	Experiment day 20	6	heated	0.0057	11.59	5.87
2	Experiment day 20	7	heated	0.0057	11.11	5.4
2	Experiment day 20	8	heated	0.0154	13.49	7.78
2	Experiment day 20	9	heated	0.0145	13.49	7.78
2	Experiment day 20	10	heated	0.0257	15.87	10.16
3	Experiment day 20	11	unheated	0.0167	13.81	8.2
3	Experiment day 20	12	unheated	0.0162	14.6	8.99
3	Experiment day 20	13	unheated	0.0142	13.49	7.88
3	Experiment day 20	14	unheated	0.024	15.08	9.47
3	Experiment day 20	15	unheated	0.0152	13.17	7.56
3	Experiment day 20	16	unheated	0.0128	13.17	7.56
3	Experiment day 20	17	unheated	0.0204	14.6	8.99
3	Experiment day 20	18	unheated	0.0212	15.4	9.78
3	Experiment day 20	19	unheated	0.0163	13.65	8.04
4	Experiment day 20	20	unheated	0.0297	15.87	10.26
4	Experiment day 20	21	unheated	0.0061	11.43	5.82
4	Experiment day 20	22	unheated	0.0101	12.86	7.24
4	Experiment day 20	23	unheated	0.0338	17.14	11.53
4	Experiment day 20	24	unheated	0.0083	11.9	6.29
4	Experiment day 20	25	unheated	0.0072	11.75	6.13
4	Experiment day 20	26	unheated	0.0098	12.38	6.77
4	Experiment day 20	27	unheated	0.0248	16.19	10.58
4	Experiment day 20	28	unheated	0.0287	16.35	10.74
7	Experiment day 20	29	unheated	0.0045	10.48	4.86
7	Experiment day 20	30	unheated	0.0089	12.06	6.45
7	Experiment day 20	31	unheated	0.0077	11.9	6.29
7	Experiment day 20	32	unheated	0.0235	15.08	9.47
7	Experiment day 20	33	unheated	0.0159	14.29	8.67
7	Experiment day 20	34	unheated	0.0353	16.67	11.05
8	Experiment day 20	35	heated	0.0012	8.8	3.08
8	Experiment day 20	36	heated	0.0037	10	4.28
8	Experiment day 20	37	heated	0.0033	10	4.28
8	Experiment day 20	38	heated	0.0026	9.7	3.98
8	Experiment day 20	39	heated	0.0033	10.5	4.78
8	Experiment day 20	40	heated	0.0035	10.4	4.68
8	Experiment day 20	41	heated	0.0039	10.3	4.58
8	Experiment day 20	42	heated	0.0046	10.9	5.18
8	Experiment day 20	43	heated	0.0114	12.7	6.98
9	Experiment day 20	44	unheated	0.0183	14.13	8.51
10	Experiment day 20	45	heated	0.0103	12.22	6.51

10	Experiment day 20	46	heated	0.006	11.43	5.71
13	Experiment day 20	47	unheated	0.0013	8.1	2.49
13	Experiment day 20	48	unheated	0.0046	10.4	4.79
13	Experiment day 20	49	unheated	0.0076	12.22	6.61
13	Experiment day 20	50	unheated	0.0116	13.17	7.56
13	Experiment day 20	51	unheated	0.0118	13.02	7.4
13	Experiment day 20	52	unheated	0.013	13.49	7.88
15	Experiment day 20	53	unheated	0.0172	13.81	8.2
15	Experiment day 20	54	unheated	0.0184	13.81	8.2
15	Experiment day 20	55	unheated	0.0542	18.41	12.8
20	Experiment day 20	56	heated	0.0063	11.27	5.55
20	Experiment day 20	57	heated	0.0128	13.49	7.78
20	Experiment day 20	58	heated	0.0072	11.59	5.87
20	Experiment day 20	59	heated	0.0149	13.17	7.46
20	Experiment day 20	60	heated	0.0321	16.67	10.95
20	Experiment day 20	61	heated	0.0105	13.02	7.3
20	Experiment day 20	62	heated	0.0267	16.35	10.63
20	Experiment day 20	63	heated	0.0105	12.7	6.98
20	Experiment day 20	64	heated	0.023	15.24	9.52
21	Experiment day 20	65	unheated	0.0155	14.13	8.51
21	Experiment day 20	66	unheated	0.0248	15.24	9.63
21	Experiment day 20	67	unheated	0.0535	18.73	13.12
21	Experiment day 20	68	unheated	0.0094	12.22	6.61
21	Experiment day 20	69	unheated	0.0274	16.19	10.58
24	Experiment day 20	70	heated	0.0034	10.13	4.41
24	Experiment day 20	71	heated	0.0035	10.63	4.91
24	Experiment day 20	72	heated	0.0057	11.38	5.66
24	Experiment day 20	73	heated	0.0053	10.88	5.16
24	Experiment day 20	74	heated	0.0119	13.5	7.78
25	Experiment day 20	75	heated	0.0024	9.38	3.66
25	Experiment day 20	76	heated	0.0039	10.5	4.78
25	Experiment day 20	77	heated	0.004	10.25	4.53
25	Experiment day 20	78	heated	0.0034	10.38	4.66
25	Experiment day 20	79	heated	0.0076	11.88	6.16
25	Experiment day 20	80	heated	0.014	13.5	7.78
26	Experiment day 20	81	heated	0.0071	12.22	6.51
26	Experiment day 20	82	heated	0.0927	21.59	15.87
28	Experiment day 20	83	heated	0.0593	19.84	14.13
28	Experiment day 20	84	heated	0.0607	19.52	13.81
28	Experiment day 20	85	heated	0.0041	10.63	4.92
28	Experiment day 20	86	heated	0.004	10.32	4.6
28	Experiment day 20	87	heated	0.0086	11.9	6.19
28	Experiment day 20	88	heated	0.033	16.51	10.79
28	Experiment day 20	89	heated	0.0183	14.6	8.89
28	Experiment day 20	90	heated	0.0327	16.67	10.95
29	Experiment day 20	91	unheated	0.0066	11.75	6.14
29	Experiment day 20	92	unheated	0.0099	12.75	7.14
29	Experiment day 20	93	unheated	0.0136	13.5	7.89
29	Experiment day 20	94	unheated	0.0053	11.13	5.51
29	Experiment day 20	95	unheated	0.0028	10.25	4.64
29	Experiment day 20	96	unheated	0.0046	10.25	4.64
29	Experiment day 20	97	unheated	0.0073	12.13	6.51

29	Experiment day 20	98	unheated	0.0182	14.88	9.26
29	Experiment day 20	99	unheated	0.0129	13.88	8.26
30	Experiment day 20	100	heated	0.0049	10.88	5.16
30	Experiment day 20	101	heated	0.0115	13.38	7.66
32	Experiment day 20	102	heated	0.0039	10.5	4.78
32	Experiment day 20	103	heated	0.0013	8.8	3.08
32	Experiment day 20	104	heated	0.0011	8.4	2.68
32	Experiment day 20	105	heated	0.005	11.8	6.08
32	Experiment day 20	106	heated	0.003	10	4.28
32	Experiment day 20	107	heated	0.0036	10.6	4.88
32	Experiment day 20	108	heated	0.0047	11.1	5.38
35	Experiment day 20	109	unheated	0.0053	11.5	5.89
35	Experiment day 20	110	unheated	0.0052	11.13	5.51
35	Experiment day 20	111	unheated	0.0034	10.5	4.89
35	Experiment day 20	112	unheated	0.0038	10.63	5.01
35	Experiment day 20	113	unheated	0.0044	10.88	5.26
35	Experiment day 20	114	unheated	0.0059	11.5	5.89
35	Experiment day 20	115	unheated	0.0043	10.88	5.26
35	Experiment day 20	116	unheated	0.0061	11.75	6.14
35	Experiment day 20	117	unheated	0.0042	10.63	5.01
35	Experiment day 20	118	unheated	0.0047	10.88	5.26
37	Experiment day 20	119	unheated	0.0034	10.5	4.89
37	Experiment day 20	120	unheated	0.0046	11.1	5.49
37	Experiment day 20	121	unheated	0.0031	10.8	5.19
37	Experiment day 20	122	unheated	0.0044	10.9	5.29
37	Experiment day 20	123	unheated	0.0022	10.1	4.49
37	Experiment day 20	124	unheated	0.0074	12	6.39
37	Experiment day 20	125	unheated	0.0052	11.5	5.89
37	Experiment day 20	126	unheated	0.0046	10.8	5.19
38	Experiment day 20	127	heated	0.007	12.38	6.67
39	Experiment day 20	128	heated	0.0074	12.38	6.66
39	Experiment day 20	129	heated	0.0086	12.63	6.91
39	Experiment day 20	130	heated	0.0081	12.63	6.91
39	Experiment day 20	131	heated	0.0046	11.25	5.53
39	Experiment day 20	132	heated	0.0052	11.75	6.03
39	Experiment day 20	133	heated	0.0023	9.9	4.18
39	Experiment day 20	134	heated	0.0029	10.9	5.18
40	Experiment day 20	135	unheated	0.006	11.63	6.01
40	Experiment day 20	136	unheated	0.0018	9.5	3.89
40	Experiment day 20	137	unheated	0.0018	9.4	3.79
40	Experiment day 20	138	unheated	0.0028	10	4.39
4	July 2	139	unheated	0.216	30.16	/
10	July 2	140	heated	0.154	31.27	/
13	July 2	141	unheated	0.098	25.08	/
13	July 2	142	unheated	0.113	27.62	/
13	July 2	143	unheated	0.118	27.46	/
15	July 2	144	unheated	0.135	28.1	/
20	July 2	145	heated	0.29	33.81	/
21	July 2	146	unheated	0.196	32.38	/
21	July 2	147	unheated	0.164	30.63	/
24	July 2	148	heated	0.062	23.49	/
26	July 2	149	heated	0.21	33.02	/

27	July 2	150	unheated	0.171	29.21	/
27	July 2	151	unheated	0.103	25.08	/
29	July 2	152	unheated	0.14	27.3	/
30	July 2	153	heated	0.23	31.9	/
32	July 2	154	heated	0.1	24.44	/

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Climate warming significantly impacts organisms, especially ectotherms. This thesis investigates how long-term whole-ecosystem warming alters fish phenotypically and genetically, as well as how these changes affect food webs. Findings from each independent study jointly point to the occurrence of evolution in response to warming in a wild fish population. The thesis emphasizes the importance of an integrative perspective when viewing the effects of climate warming on populations and communities to achieve a more general understanding.

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Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

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