# Ecological performance of pet-store goldfish (*Carassius auratus*) under elevated temperature and chloride levels

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## ABSTRACT

Anthropogenic stressors can affect the invasion success and impact of introduced fishes in freshwater ecosystems. Predicting the performance of species subjected to multiple stressors is needed to assess ecosystem vulnerabilities and prioritize species likely to be disruptive. This thesis explores how the ecological performance of cultivated pet store goldfish (Carassius auratus) is affected by exposure to climate warming and chloride pollution via road salt application-two stressors that are increasingly significant in north temperate urban ponds. The goldfish, native to China, is a global invader that is reportedly increasing its distribution and abundance in ponds, lakes and wetlands in Canada, most notably in the Great Lakes basin. A review of published studies indicates that goldfish have a broad environmental tolerance across life stages that is enhanced through acclimation; however, data on tolerance to multiple cooccurring stressors are lacking, which can impede predictive risk assessment. To understand resource consumption dynamics and thermal tolerance of cultivated goldfish under climate warming scenarios and chloride pollution, I used a series of experiments to measure functional response and critical thermal maxima of goldfish obtained from a pet supplier. The fish were acclimated to a combination of temperature and chloride regimes that reflect current and projected summer temperatures in the Great Lakes basin as well as current chloride levels in Canadian urban ponds. I tested predictions that superior foraging abilities and thermal tolerance would increase with acclimation to temperatures and chloride levels that closely match the species' reported optimum (the Environmental Matching Hypothesis). As predicted, resource consumption and CTmax increased with acclimation to higher temperatures that overlap with their reported optimum. Contrary to my prediction, results varied with different temperaturechloride combinations. Overall, my findings suggest that invasive species risk assessments should be informed by incorporating multiple context dependencies at ecologically relevant thresholds.

## RÉSUMÉ

Les facteurs de stress anthropogénique peuvent affecter le succès d'invasion et l'impact des poissons introduits dans les écosystèmes d'eau douce. Il est nécessaire de prédire la performance des espèces soumises à de multiples facteurs de stress pour évaluer les vulnérabilités des écosystèmes et prioriser les espèces susceptibles d'être perturbatrices. Cette thèse explore comment la performance écologique des poissons rouge d'animalerie cultivés (Carassius auratus) est affectée par l'exposition au réchauffement climatique et à la pollution par les chlorures due à l'application de sel de voirie - deux facteurs de stress qui sont de plus en plus significatifs dans les étangs urbains du nord tempéré. Le poisson rouge, originaire de Chine, est un envahisseur mondial dont la répartition et l'abondance augmentent dans les étangs, les lacs et les zones humides du Canada, notamment dans le bassin des Grands Lacs. Une analyse des études publiées indique que les poissons rouges ont une large tolérance environnementale à tous les stades de leur vie, renforcée par l'acclimatation. Cependant, les données sur la tolérance à de multiples facteurs de stress cooccurrents manquent, ce qui peut nuire à l'évaluation prédictive des risques. Pour comprendre la dynamique de la consommation des ressources et la tolérance thermique des poissons rouges cultivés dans le cadre de scénarios de réchauffement climatique et de pollution par les chlorures, j'ai utilisé une série d'expériences pour mesurer la réponse fonctionnelle et les maxima thermiques critiques (CTmax) de poissons obtenus auprès d'un fournisseur d'animaux de compagnie. Les poissons ont été acclimatés à une combinaison de régimes de température et de chlorure reflétant les températures estivales actuelles et projetés dans le bassin des Grands Lacs ainsi que les niveaux actuels de chlorure dans les étangs urbains canadiens. J'ai testé les prédictions selon lesquelles les capacités supérieures de recherche de nourriture et la tolérance thermique augmenteraient avec l'acclimatation à des températures et des niveaux de chlorure qui correspondent étroitement à l'optimum de croissance de l'espèce (l'hypothèse de l'adaptation à l'environnement). Comme prévu, la consommation de ressources et le CTmax augmentent avec l'acclimatation à des températures plus élevées chevauchant leur optimum déclaré. Contrairement aux prévisions, les résultats varient en fonction des différentes combinaisons température-chlorure. Dans l'ensemble, mes résultats suggèrent que les évaluations des risques liés aux espèces envahissantes devraient être éclairées par l'incorporation de multiples dépendances contextuelles à des seuils écologiquement pertinents.

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## CONTRIBUTION OF AUTHORS

This thesis was completed under the supervision of Prof. Anthony Ricciardi at McGill University. He will be a co-author of the submitted manuscript. All experiments, protocol development, statistical analysis and literature review were completed by me. Prof. Ricciardi provided help with research ideas, feedback and editing of my manuscript.

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### GENERAL INTRODUCTION

A biological invasion occurs when a species is introduced to, and establishes a sustainable population, in a new environment beyond its historical range; such species, whether established or not, are called 'non-native', 'nonindigenous', or 'alien' (Ricciardi, 2013). In addition to driving biodiversity loss, invasions can alter ecosystem functioning, interfere with recreational and commercial fisheries, impact local economies, and affect human health (Bertelsmeier & Keller, 2018; Pyšek et al., 2020; Ricciardi et al., 2017; Sala et al., 2000; Simberloff et al., 2013). Species introduction can occur via natural processes such as storms, and floods; however, human activities are the predominant driver of introductions causing rates of invasion to reach unprecedented levels (Ricciardi, 2007), particularly in large freshwater systems (i.e. Laurentian Great Lakes; Ricciardi, 2006). Activities including shipping, aquaculture, canal construction, sewage practices, water diversion, recreational fishing, live seafood trade and aquarium release contribute to the successful establishment of species (Chapman et al., 2020; Lodge et al., 1998; Ricciardi, 2006; Rixon et al., 2005; Sala et al., 2000).

The pet trade, specifically aquarium species, is a major source of aquatic invaders in freshwater environments (Dickey et al., 2023; Evers et al., 2019; Padilla & Williams, 2004). This ever-expanding industry, supporting \$25 billion annually, is highly unregulated despite the threat it poses to ecosystems (Padilla & Williams, 2004). With species being sold online, obtaining diverse aquatic pets has become increasingly accessible, even for some now illegal species (Borges et al., 2021). Many pets are intentionally released by owners unaware of responsible disposal methods; a customer Montreal survey revealed that 6.98% of pet fish owners have reported releasing at least one fish with reasons for disposal being either the pet's aggressive behaviour, large size, illness, rapid reproduction or other (Gertzen et al., 2008). A total of ~10,000 fish are estimated to be released annually in the area of Montreal and the upper St Lawrence River (Gertzen et al., 2008).

Often, more popular ornamental species are successful invaders because they are involved in more release events, hence increasing the likelihood of establishment (Duggan et al., 2006). This is clear when correlating the most readily available freshwater aquaria species and those widespread in the Great Lakes, such as goldfish (*Carassius auratus*) and koi (*Cyprinus carpio*) (Rixon et al., 2005). Species may become popular in the pet trade not only for their aesthetics but

also because they are tolerant species, making them easier to breed and cultivate and for owners to care for them. This could create a selective filter for species that are resistant to environmental stressors when released, giving them a competitive advantage against more sensitive taxa. Aquatic pets like goldfish are often initially released into urban water bodies owing to their proximity to populated areas (Copp et al., 2005). Urban waterways are subjected to multiple anthropogenic stressors (Brans et al., 2018) which could select hardy individuals adapted to human-disturbed environments, potentially conferring upon them the ability to invade other habitats that have become or are in the process of becoming disturbed (cf. Anthropogenically Induced Adaptation to Invade Hypothesis; Hufbauer et al., 2012). Subsequent dispersal of pets from urban to wild waterbodies poses a risk of enhanced competitiveness and impacts as wild waterbodies are increasingly subject to stressors experienced in urban environments, although less acutely and at a slower pace (Philip et al., 2022). Data on the tolerance of pet species to urban stressors are therefore needed to inform risk assessment. With respect to the field of invasion science, there is a need to develop better predictions of how the behaviour of non-native species varies in relation to changing environmental conditions, including most notably climate change (Ricciardi et al., 2021).

The goal of this thesis was to identify how urban stressors, specifically climate warming and salt pollution via de-icing application, affect the performance and physiology of a commonly released pet store fish, the common goldfish (*Carassius auratus*). My specific objectives were to (1) synthesize data on goldfish tolerance and resistance to particular anthropogenic stressors (Chapter 1) and conduct experiments to reveal how exposure of goldfish to potentially interactive stressors (warming  $\times$  chloride) affects (2) their feeding efficiency as indicated by their functional response (Chapter 2) and (3) their upper thermal tolerance including their critical thermal maximum (Chapter 3). As such, my thesis comprises a literature review (Chapter 1), two original data chapters (Chapters 2 and 3), and a general conclusion. The two data chapters are written in manuscript format, as they are intended to ultimately be submitted to peer-reviewed journals.

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## CHAPTER 1: A LITERATURE REVIEW OF THE INFLUENCES OF ANTHROPOGENIC STRESSORS ON GOLDFISH (*CARASSIUS AURATUS*) PERFORMANCE AND TOLERANCE

## Introduction

#### Aquatic Invasions Under Urbanization

Aquatic invaders are increasingly subjected to multiple co-occurring environmental stressors. Landscape modifications due to increasing urbanization have documented impacts on freshwater ecosystems (Chadwick et al., 2006; Groffman et al., 2014; Paul & Meyer, 2001). Human-provoked environmental changes affect the establishment, population growth and impact of introduced non-native species (e.g., Hufbauer et al., 2012; Zimmermann et al. 2014; Putnam et al., 2020). The Anthropogenic Induced Adaptation to Invade hypothesis proposes that contemporary adaptation to human-disturbed habitats confers traits such as greater tolerance of physiological stressors (Hufbauer et al., 2012) that predispose species to invade human-altered (e.g. urbanized) habitats in other regions if given the opportunity. Thus, introduced species that have evolved under urban contexts, termed "urbanized invaders," may have an advantage under globally changing conditions (Borden & Flory, 2021).

Owing to their insularity, extensive exploitation for recreation and industry, and proximity to human populations, freshwater ecosystems are highly invaded (Bernery et al., 2022; Ricciardi & MacIsaac, 2011). In the Laurentian Great Lakes, for example, human activities have contributed to the successful establishment of over 180 non-native species (Ricciardi, 2006). Since the Industrial Revolution, accelerating development and climate change have influenced the establishment, abundance, spread and impact of non-native species (Christensen et al., 2020; Iacarella et al., 2015; Mandrak, 1989; Rahel & Olden, 2008). Understanding a non-native species' response to multiple simultaneous environmental changes is critical to understanding spatiotemporal variation in their invasion success and ecological impacts. In this review, I first outline major disturbances experienced in freshwater ecosystems in North America and then summarize data on how the goldfish—a major North American invader widely distributed in urbanized areas—responds to these changing conditions.

#### Influences of anthropogenic stressors on freshwater ecosystems

Land modification has affected over three-quarters of the Earth's surface over the last millennium (Winkler et al., 2021) through deforestation, agriculture, expanding infrastructure, and urban development (Luyssaert et al., 2014; Winkler et al., 2021). Land changes and associated activities increase concentrations of pollutants (including chloride), nutrient loading, and rates of hypolimnetic oxygen consumption in inland waters (Dugan et al., 2017; St-Hilaire et al., 2016; Tellier et al., 2022). For example, intensive farming is a source of phosphorus and nitrogen pollution exceeding water quality guidelines (St-Hilaire et al., 2016).

The effects of climate change are amplified through urbanization due to artificial aesthetics and impervious surfaces (i.e. urban heat island effect; Touchaei & Wang, 2015; Wang & Akbari, 2016). Water bodies found in these habitats experience greater temperature fluctuations (Brans et al., 2018). Furthermore, warmer water and excess nutrients can contribute to hypolimnetic hypoxia (Tellier et al., 2022).

Urban waterways are also subjected to alterations in water chemistry. Changes to major ion composition (e.g., Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, NO<sub>3</sub><sup>-</sup>, SO, Br<sup>-</sup>) and pH impact lake dynamics, biochemical processes and community composition (Hintz et al., 2022; Kaushal et al., 2017; Kaushal et al., 2021; Kaushal et al., 2018). This is a global reality for lentic waterways (Zhang et al., 2022) but is particularly evident in regions with heavy snowfall where de-icing salts, typically sodium chloride (NaCl), are applied in the winter and are major components of runoff from impervious land cover (Dugan et al., 2017; Fournier et al., 2021). Elevated levels of chloride far exceeding the Canadian Water Quality Guideline for the Protection of Aquatic Life (120 mg/L (CCME, 2011) have been observed in waterways near urban areas or major highways (Dugan et al., 2017).

#### Goldfish (Carassius auratus): a model invader in the Anthropocene

The common goldfish (*Carassius auratus*), a popular household pet, is ubiquitous in the wild and found on all continents except Antarctica. Native to central China, this cyprinid fish was introduced to North America as early as the 17th century but only became widespread in the past ~100 years (Courtenay & Stauffer, 1990; Halas et al., 2018). It has successfully established populations throughout the Great Lakes basin (Mills et al., 1993; Richardson et al., 1995) and in all Canadian provinces. Goldfish typically occupy lentic waters, slow-lotic systems, and often shallow waters with submerged vegetation (Lorenzoni et al., 2007). When released from captivity and placed in unrestricted bodies of water, Goldfish can reach large body sizes, nearing 45cm, and live long lives; 6 years+ (Lorenzoni et al., 2007). They are aggressive generalist feeders, eating both plants and protein via their "suck, spit, and pick" foraging method (Richardson et al., 1995). The broad distribution and local abundance of goldfish can be credited to physiological tolerances allowing the species to thrive even in stressed habitats (Rahel & Olden, 2008). Goldfish introductions are directly attributable to human activity, with the primary source of introduction being deliberate aquarium release (Courtenay & Stauffer, 1990); the presence of introduced goldfish in urban ponds is correlated with the pond's distance from the nearest road, footpath, and habitation (Copp et al., 2005). Non-native populations have become increasingly established in temperate regions over the past few decades, and some populations have exploded exponentially in recent years for mysterious reasons (Boston et al., 2024).

Perhaps due to its domestication and simpatico aesthetic, there is a prevailing public perception of the wild presence of goldfish as being benign. In contrast to this perception, studies have documented various ecosystem and economic impacts (Dickey et al., 2022; Richardson et al., 1995). Impact-scoring studies have revealed that goldfish are high-risk invaders that merit management intervention (Dickey et al., 2022; Van Der Veer & Nentwig, 2015). Goldfish compete with native species for resources (Magalhães & Jacobi, 2013; Roy, 1992), transmit diseases (Faillace et al., 2017), hybridize with certain other fishes (Halas et al., 2018; Hanfling et al., 2005), reproduce rapidly through gynogenesis (Lorenzoni et al., 2007), and re-engineer ecosystems by altering water clarity and disturbing sediments to the detriment of resident plants and animals (Morgan & Beatty, 2007; Richardson et al., 1995). Their broad environmental tolerance to temperature (Ford & Beitinger, 2005), pH (Nadermann et al., 2019), salinity (Schofield et al., 2006), hypoxia (McNeil & Closs, 2007), desiccation (Nakamura, 1995), and water turbulence (Nadermann et al., 2019) renders many disturbed ecosystems suitable for their establishment and confers them an advantage over less tolerant resident species.

### Environmental tolerance and performance of Carassius auratus

Using separate combinations of key words (Table 1.1), I searched the biological literature on online databases (ISI *Web of Science*) for studies that reported on the effects of anthropogenically induced water chemistry changes on the success of goldfish. Papers found in relevant bibliographies that were not detected in the original search were also included. A total of 698

were revealed in the search results, of which 47 were deemed relevant (Table 1.1). I eliminated studies with ambiguous species identifications, whereby various authors use the Latin name for the common goldfish (*Carassius auratus*) but refer to other congeneric species (i.e., Crucian carp, Prussian carp).

#### Temperature

Goldfish are a thermally tolerant species capable of withstanding temperatures ranging from 0.3°C to 44.7°C (Ferreira et al., 2014; Ford & Beitinger, 2005). Three types of indicators with standardized methods are typically used to evaluate temperature tolerance: (1) Critical Thermal Maxima (CTmax); (2) Critical Thermal Minima (CTmin); and (3) Upper Lethal Temperature (ULT). These metrics are derived from experiments that acclimatize a fish to a certain temperature before exposing it to continuous warming/cooling until it loses equilibrium (CTmax and CTmin, respectively) or death occurs (ULT), both denoting endpoints for thermal tolerance (Bennett & Beitinger, 1997; Fry, 1947). Literature data for these metrics reveal a consistent linear relationship between acclimation temperature and the capacity of goldfish to withstand acute temperature shifts (Figure 1.1). Context dependent variation is evident, but the influence of other stressors has only been quantified in one published study (Weatherley 1970), along with the work presented in this thesis (Chapter 3). Weatherley (1970) revealed that superabundant oxygen conditions (up to ~5atm) can improve thermal tolerance (Table 1.2). In Chapter 3, I show that while temperature acclimation linearly increases CTmax, the addition of chloride can either enhance or dampen thermal tolerance (Table 1.2; Chapter 3). Fish size has been reported to be an influential co-variate, with smaller fish generally displaying higher tolerance, although the nature of the relationship varies with species (Recsetar et al., 2012). Only 4 of 7 of the thermal tolerance studies I reviewed reported body metrics, complicating a consensus on the relationship between the size and tolerance of goldfish (Table 1.2). There is no detectable relationship between upper thermal tolerance and goldfish mass; individuals of both small and large mass tolerate similar upper limits of ~40°C (Ferreira et al., 2014; Khieokhajonkhet et al., 2023; Yanar et al., 2019). Upper thermal tolerance in freshwater fishes has been reported to vary little with life stage, from fry to adult (Recsetar et al., 2012); however, we cannot make a similar conclusion for goldfish as only 2 of 7 studies specified the life stage, both using juveniles. Only one study investigated intersexual differences and found no effect of sex of thermal capabilities (Yanar et al., 2023) (Table 1.2).

Thermal optima for goldfish were determined based on published performance metrics: growth, feeding and digestion, locomotion, reproduction, and development (Table 1.3). Growth performance for larval stages (average mass: 0.00115-0.0002 g) is optimal at temperatures 27-28°C, as revealed through specific growth rate measures (Imanpoor et al., 2012; Kestemont, 1995). For fish of larger size (2.45g) or juvenile stage, high growth was observed over a wider range of temperatures; between 25°C (Audige, 1921) and 27–34°C (Khieokhajonkhet et al., 2023). The growth rate and food consumption optima overlap, whereby food consumption rates are highest at 25–30°C (Chen et al., 2019; Nadermann et al., 2019; Rozin & Mayer, 1961), the digestion process is shortest at 25°C (Pang et al., 2011), and food conversion efficiency is optimal at 27-30°C (Khieokhajonkhet et al., 2023). Locomotor performance varies depending on the metric used. Goldfish escape speed (C-start metric: startle escape behaviour whereby a fish begins by bending into a "C" curve) was best at lower temperatures of 20°C (Johnson et al., 1998) and 18°C (Preuss & Faber, 2003). The avoidance ability of goldfish is possible within a range of 3-42°C, with optimal avoidance success maintained along a thermal gradient of 20– 30°C (Hoyland et al., 1979). The critical swimming speed (Ucrit) of juveniles fell in a higher range of temperatures, 20–30°C (Fry & Hart, 1948). Goldfish Ucrit increased with acclimation temperature, and their thermal history allowed them to perform better when tested at higher temperatures, outside the acclimation range (Fry & Hart, 1948). The hatching performance of embryo life stages is in the range of 18 to 30°C (Motta et al., 2023), although most studies report optimum within the middle of the range (Battle, 1940; Urushibata et al., 2019; Wiegand et al., 1988). Temperatures above 34°C result in lethal effects and 100% abnormalities (Motta et al., 2023). Goldfish usually spawn in early/mid-spring, with ovulation being triggered at 12-20°C (Stacey & Liley, 1974), corresponding with their reported optimum (measured using gonad secretion) at temperatures of 17-30°C (Gillet et al., 1977).

### Oxygen

Critical oxygen partial pressure (Pcrit) is used as a proxy for oxygen tolerance and is defined as the oxygen level where an animal's oxygen uptake is dependent on environmental levels due to an inability to maintain a stable rate of oxygen uptake (Seibel et al., 2021). A comprehensive review of the Pcrit goldfish research revealed that goldfish are hypoxic-tolerant species, reported to be able to tolerate low oxygen conditions (1-3.38kPa) and anoxic conditions (0kPa) for a sustained period (10 hours) (Table 1.4). Hypoxia acclimation history, exercise acclimation and

variation in trial length (i.e. time to induce anoxia conditions) influenced tolerance capability, with acclimation and longer trial duration (i.e. acute acclimation) improving hypoxia tolerance. Aquatic surface respiration is often noted in Pcrit trials as this behaviour (fish rising to the surface and skimming the water-air interface to get oxygen) reveals adaption potential. Goldfish have been shown to display such hypoxia-avoidance strategies staying at oxygen levels of 0.84mg/L, with peak ASR at levels as low as 0.2 mg/L (McNeil & Closs, 2007).

While normoxic oxygen levels (5-8mg/L) are needed to support metabolic needs and fitness, thus can be deemed "optimal", there is a lot that can be said regarding the performance of goldfish at suboptimal levels, specifically in terms of their locomotive and reproduction. In terms of swimming performance, low oxygen conditions cause significant decreases in Ucrit values: a 44% decrease with oxygen decrease from 8 to 1mg/L, with optimal swimming performance occurring at oxygen levels of 8mg/L (Zhang et al., 2012). However, acclimation to hypoxia buffers this effect, causing significantly higher Ucrit values, relative to un-acclimated groups when tested in hypoxia (Fu et al., 2011) or normoxia (Thoral et al., 2022). In some instances, fish acclimated to hypoxia performed just as well as ambient acclimated groups; however, it is important to note that here swimming effort was tested at higher temperatures (Kutty, 1968). Lower temperatures also have this buffering effect, by allowing fish to withstand lower oxygen concentrations before the occurrence of swimming failure (15°C: 0.44 ppm of O2 at 25cm/sec vs 30°C: 1.45ppm of O2 at 25cm/sec) (Smit et al., 1971). Only one study was found relating to the impact of hypoxia on reproductive performance. It was shown that exposure to hypoxia impaired reproduction by reducing sperm count and impairing stages of gonad development (Bera et al., 2017).

#### Ions and alkalinity

Goldfish exhibit a remarkable capacity for ion tolerance. Most frequently reported is their salinity (NaCl) tolerance (10/13 studies, Table 1.5), whereby goldfish can withstand salinities up to 30ppt, measured via survival rates (Wang et al., 2023). Variation in tolerance is seen along life stages, with more limited survival rates during the early years and exposure time (Table 1.5). During acute exposure, goldfish can survive high concentrations, up to 30ppt for 20 minutes, however, increased exposure (30min) results in large mortalities (Wang et al., 2023). Through chronic exposure and thus adaptation potential, goldfish can withstand salinities up to 16ppt

without compromising survivorship (Küçük, 2013) (Table 1.5). Goldfish eggs were reported to be unaffected by salinities up to 8ppt; however, after hatching, tolerance declines, with fry not surviving sanities of 8ppt (Murai & Andrews, 1977). Larvae life stages can withstand salinity of 12ppt (Imanpoor et al., 2012), juvenile, and adult salinity tolerances vary among populations, with a reported range of 10-30ppt (Altinokand & Grizzle, 2001; Küçük, 2013; Schofield et al., 2006; Wang et al., 2023).

The effect of salinity on various performance metrics (locomotion, growth and feeding) was used to determine the optimum. In both acute (<14 days) and chronic (14 days) trials, salinities 0-3ppt were the most adaptable whereby goldfish were the most active and displayed the best escape ability (Lawson & Alake, 2010). While salinities above were tolerated (4–10ppt) they caused erratic movement or weaker threat responses (Lawson & Alake, 2010). The salinity optimum for goldish swimming performance might be dependent on the time of day; freshwater (0ppt) caused optimal diurnal activity, whereas salinity has no impact on nocturnal activity (Luz et al., 2008).

Optimal salinity levels for growth varied among studies. For juvenile goldfish, the consensus was that salinities ranging from 0-1 ppt have growth-enhancing effects (Altinokand & Grizzle, 2001; Canagaratnam, 1959; Lawson & Alake, 2010). Higher salinities have mixed effects either enhancing growth (6ppt and 16ppt; Küçük, 2013; Luz et al., 2008) or having adverse or no effect effects (6-10ppt; Lawson & Alake, 2010; Luz et al., 2008). However, in general, specific growth rate and weight gain generally decreased with increasing salinity (Küçük, 2013; Luz et al., 2008). For larval life stages, the optimal range in terms of growth is 0-6ppt in temperatures ranging from 23-27°C (Imanpoor et al., 2012). Feeding rate follows a similar trend where the optimum is reported to be in the range of 1-3ppt (Lawson & Alake, 2010; Luz et al., 2008), although another study found no difference up to 16ppt (Küçük, 2013).

Apart from reports on salinity, my review found studies of copper pollution tolerance and performance (2 of 13 studies), whereby increases in copper concentration (> 0.4mg/L) and exposure time increase mortality and larval deformity rate with acute exposure (Kong et al., 2013). Swimming performance (Ucrit) remained unaffected by copper pollution exposure with acute and chronic (7 days) exposure to 0.34-0.84µM. (Moyson et al., 2016).

Finally, in terms of pH effects of goldfish performance, feeding rate was optimal at pH of 7.5 compared with a pH of 5-6 (Nadermann et al., 2019).

#### Literature Gaps and Conclusions

Despite a substantial body of research on goldfish tolerance and performance, several gaps are evident in the literature. Tolerance and performance can vary with age, whereby earlier life stages are typically more vulnerable and have lower thresholds (i.e. see Table 1.5 on Salinity). Less than half of all reviewed studies (20 of 47) reported fish age (Table S1.1). Furthermore, although fish length and weight are standard measures that can easily be reported for all studies and are known to greatly affect the performance and tolerance of fishes including goldfish (Chapter 1, Avlijaš et al., 2022), over 20% (10/47) of studies failed to report these body metrics.

Most published studies evaluate the effects for a single stressor in isolation. Those few studies that did investigate simultaneous stressors typically incorporated temperature as a factor (Imanpoor et al., 2012; Weatherley, 1970). The reality of invasion is that goldfish will be subjected to multiple stressors in urban environments. To avoid naïve or inaccurate risk assessments, there is a critical need to gather data on these potentially interacting stressors on goldfish proliferation and impact.

Finally, much of the literature has concentrated on commercially sourced goldfish and conducted experiments in the laboratory (84%, Table S1.1). This does not accurately reflect natural habitats and ecological interactions, thus impairing the accurate management of invasive populations. Invasive populations in the wild should be compared with cultivated populations to investigate environmental adaption or selection after pet release.

In summary, common goldfish display a remarkable tolerance to temperature, salinity and oxygen and an adaptability to perform under changing conditions. This plasticity contributes to their global invasion success. While the generalization of findings is complicated by variation in studies, life stages and methodologies, we can conclude that 1) acclimation enhances tolerance; 2) acclimation to a stressor can heighten goldfish performance under stressful conditions; and 3) more data from invasive populations, multiple context dependencies and field experiments are required to inform risk assessment.

## Tables and Figures

Table 1.1. Summary of search terms, search results and number of studies $(n = 47)$ used for the
literature review categorized by water chemistry topic.

Topic	Key Words	Common Keywords	Search	Papers
			Results	retained
Temperature	Temperature OR thermal OR warming	Goldfish OR "Carassius auratus"	218	25
Oxygen	Anoxia OR hypoxia OR "dissolved oxygen" OR oxygen	OR "Gold fish"	222	11
Ions and Alkalinity	Ion* OR conductivity OR salini* OR alkalin* OR pH OR "specific conductance" OR calcium OR chloride OR salt	tolerance OR optimum OR resistance	258	13 *two studies also used in temperature
Total	·		698	47

\*Inclusion criteria: studies that use laboratory experiments, field studies and controlled conditions. Studies quantifying measures of performance and tolerance (see results) with measurable outcomes were used. Studies focusing on biochemistry aspects of fish biology were excluded.

**Table 1.2**. Summary of the studies (n=8) used to investigate the thermal tolerance of goldfish (*Carassius auratus*). When studies provided a range of values for body metrics, here we report the average of all values. If contexts were tested, we report the "control" tolerance value representing no context, followed by the highest value and the test conditions *displayed in italics*.

Tolerance Metric	End point temp, (acclimation temp) (°C)	Life Stage	Mean Weight	Study Context	Study
CTmin	4.67 (24)	NA	16.47	Sex differences: no significant difference between sexes	(Yanar et al., 2023)
	2.55 (20)	NA	5.74	NA	(Yanar et al., 2019)
	0.3 (5)	NA	NA	NA	(Ford & Beitinger, 2005)
CTmax	43.6 (35)	NA	NA	NA	
	43.83 (34)	NA	2.45	NA	(Khieokhajonkhet et al., 2023)
	40.14 (24)	NA	16.47	Sex differences: no significant difference between the sexes	(Yanar et al., 2023)
	44.7 (28)	NA	3.3	NA	(Ferreira et al., 2014)
	40 (26) 40.4 (26), context of 6ppt Cl-	NA	15.75	Chloride: at varying acclimations, chloride can enhance or dampen thermal performance	Chapter 2: Claus et al., 2024
ULT	41 (35)	Juvenile	NA	NA	(Fry et al. 1942 (unpublished), as cited in Fry & Hart, 1948)
	38.2 (27) 40.4 (27), context of 14atm	Juvenile	NA	Oxygen: Higher amounts of oxygen were associated with higher ULT until a plateau at 2-4atm for all acclimation temperatures	(Weatherley, 1970)

**Table 1.3.** Summary of the studies (n=18) used to investigate the thermal optimum of goldfish (*Carassius auratus*) based on various performance metrics. When studies provided a range of values for body metrics, here we report the average of all values. We distinguish between long vs short-term studies, where the latter is anything less than a week of temperature acclimation.

-	1				1		
Performance	Measurement	Optimum	Life	Mean	Mean	Chronic	Study
Metric		Temperature	Stage	Weight	Length	(>1week)	
		(°C)		(g)	(cm)	vs acute	
Growth	Specific	27-34	NA	2.45	NA	Chronic	(Khieokhajonkhet
	Growth Rate						et al., 2023)
		28	Newly	0.00115	NA	Acute	(Kestemont,
			hatched				1995)
			Larvae				
		24-28	10-day	2.05	NA	Acute	(Kestemont,
			old				1995)
			larvae				, ,
		23-27	Larvae	0.0002	NA	Chronic	(Imanpoor et al.,
							2012)
Feeding	Feed	27-30	NA	NA	NA	Chronic	(Khieokhajonkhet
	conversion						et al., 2023)
	efficiency/rate	23-27	Larvae	0.0002	NA	Chronic	(Imanpoor et al.,
	-						2012)
		24-28	Newly	0.00115	NA	Acute	(Kestemont,
			hatched				1995)
			Larvae				,
	Digestion rate	25	Juvenile	8.5	NA	Chronic	(Pang et al.,
	C C					temp	2011)
						acclimation,	,
						acute trial	
	Food Intake	25-30	NA	16.8	NA	Chronic	(Nadermann et
							al., 2019)
		28	NA	31	NA	Acute	(Chen et al.,
							2019)
		28	NA	31	NA	Chronic	(Chen et al.,
							2019)
		28	NA	NA	9	Chronic	(Jones, 1984)
		25	NA	55	9.5	Chronic	(Rozin & Mayer,
						acclimation	1961)
						to 25°C,	,
						acute	
						temperature	
						shift to	
						15°C, 10-	
						day trial	
Locomotor	C-start	20	NA	7.7	NA	Chronic	(Johnson et al.,
						acclimation	1998)
						to 20°C,	
						acute to	
						5°C, acute	
						trial	

		18	NA	NA	11.5	Chronic acclimation to 18°C, acute to 8°C, acute trials	(Preuss & Faber, 2003)
	Ucrit	25	Juvenile	NA	NA	Chronic temp acclimation, acute trial	(Pang et al., 2011)
		28-30	Juvenile	NA	NA	Chronic acclimation, acute trial	(Fry & Hart, 1948)
	Avoidance ability	20-30	NA	NA	NA	Acute	(Hoyland et al., 1979)
Development	Hatching	20-24	Embryo	NA	NA	Acute	(Urushibata et al., 2019)
		18-30	Embryo	NA	NA	Acute	(Motta et al., 2023)
		22	Embryo	NA	NA	Acute	(Wiegand et al., 1988)
		24-28	Embryo	NA	NA	Acute	(Battle, 1940)
Reproduction	Gonad secretion	17-30	Adult (3-4 years)	NA	NA	Chronic	(Gillet et al., 1977)

**Table 1.4.** Summary of the studies (n=5) used to investigate the hypoxia and anoxia tolerance of goldfish (*Carassius auratus*) based on various tolerance metrics. When studies provided a range of values for body metrics, here we report the average of all values. Units of oxygen were converted to kPa for uniformity. Context of the studies is reported here to distinguish between acclimated population, trial lengths and the incorporation of multiple dependencies.

Tolerance Metric	Measurement	Tolerance	Trial Temp	Life Stage	Mean Weight	Study Context	Study
			(°C)	C	(g)		
Anoxia	Penultimate oxygen tension (PO2)	No LOE recorded after 1h exposure	12	NA	4.26	No acclimation	(Dhillon et al., 2013)
		Withstand anoxia for 8h	20	NA	305.6	No acclimation	(van Ginneken et al., 1996)
	Critical Oxygen Tension (Pcrit)	Withstand anoxia for 10h	20	3 years	100	No acclimation	(van den Thillart et al., 1976)
Нурохіа		3.38 kPa	12	NA	4.26	No acclimation	(Dhillon et al., 2013)
		1.55 kPa	12	Juvenile	6.08	Hypoxia acclimated (0.3mg/L)	(Fu et al., 2011)
		1.88 kPa	12	Juvenile	6.08	Exercise acclimated (70% Ucrit)	(Fu et al., 2011)
		3.062 kPa	12	Juvenile	6.08	No acclimation	(Fu et al., 2011)
		1 kPa	17	NA	2.87	Long- duration trial (480 min), No acclimation	(Regan & Richards, 2017)
		2.5 kPa	17	NA	2.87	Short- duration trial (24 and 84 min), No acclimation	(Regan & Richards, 2017)

**Table 1.5.** Summary of the studies (n=7) of the studies used to investigate the salinity (NaCl) tolerance of goldfish (*Carassius auratus*) based on survival. When studies provided a range of values for body metrics, here we report the average of all values. Units of salinity were converted to ppt for uniformity.

Highest salinity	Highest salinity	Life stage	Acute or	Average	Study
tolerated (ppt),	(ppt) causing		chronic (>1	Weight (g)	
% surviving	100% survival		week)		
15, 61%	10	NA	Acute (72h)	22.7	(Schofield et
					al., 2006)
15,8%	5	NA	Chronic (30	36.2	(Schofield et
			days)		al., 2006)
30, 100%	30	NA	Acute (20min)	28.15	(Wang et al.,
					2023)
5,40%	3	Juvenile	Chronic (14	107.5	(Lawson &
			days)		Alake, 2010)
12, 100%	12	Larvae	Chronic (45	0.005	(Imanpoor et
			days)		al., 2012)
10, 100%	10	NA	Acute (96	5.53	(da Silva et al.,
			hours)		2021)
5,75%	0	NA	Chronic (21	5.53	(da Silva et al.,
			days)		2021)
20, 81.25%	16	NA	Chronic (20	8.99	(Küçük, 2013)
			days)		
6, 7%	**89% at 0ppt	Egg	Chronic (3	0.283	(Murai &
		-	weeks)		Andrews,
					1977)

\*\*100% survival not reported, so we report top survival



**Figure 1.1.** A scatter plot showing the effect of acclimation temperature (y axis) on various metrics of thermal tolerance measuring final temperature (x-axis) distinguished by each facet: (a) Critical Thermal Minima, (b) Critical Thermal Maxima, (c) Upper Lethal Temperature. The jitter (height= 1, width = 1) function in ggplot2 was applied to the graph to visualize overlapping points. The coefficient of determination ( $R^2$ ) for each is (a) 0.826, (b) 0.862 and (c) 0.952. N=8 studies were used (my work (Claus et al., 2024) and one unpublished work).

## Supplementary Material

**Table S1.1.** Summary of all the studies (n=47) used for the literature review for each major topic investigated (temperature, oxygen, or ion), distinguishing studies that report on life stage, body metrics and fish source. When studies provided a range of values for body metrics, here we report the average of all values.

#	Торіс	Life Stage	Weight (g), Length (cm)	Source	Study
1	Temperature	NA	15.75, 7.72	Commercially	Unpublished Claus et al., 2024
2	Temperature	NA	3.3, NA	Commercially	(Ferreira et al., 2014)
3	Temperature	NA	NA	Commercially	(Ford & Beitinger, 2005)
4	Temperature	Juvenile	NA	NA	(Fry et al. 1942 (unpublished), as
					cited in Fry & Hart, 1948)
5	Temperature	NA	2.45, NA	Commercially	(Khieokhajonkhet et al., 2023)
6	Temperature	Juvenile	NA	Commercially	(Weatherley, 1970)
7	Temperature	NA	5.74, NA	Commercially	(Yanar et al., 2019)
8	Temperature	NA	16.47, NA	Commercially	(Yanar et al., 2023)
9	Temperature	Larvae	0.0015-0.205,	Commercially	(Kestemont, 1995)
	·		NA	-	
10	Temperature	Larvae	0.0002, NA	Lab	(Imanpoor et al., 2012)
	& Ion				
11	Temperature	Juvenile	8.5, NA	NA	(Pang et al., 2011)
12	Temperature	NA	16.8, 12	NA	(Nadermann et al., 2019)
	& Ion				
13	Temperature	NA	NA	NA	(Audige, 1921)
14	Temperature	NA	31,NA	Commercially	(Chen et al., 2019)
15	Temperature	NA	NA, 9	Commercially	(Jones, 1984)
16	Temperature	NA	55, 9.5	Commercially	(Rozin & Mayer, 1961)
17	Temperature	NA	NA	Commercially	(Johnson et al., 1998)
18	Temperature	NA	NA,11.5	Commercially	(Preuss & Faber, 2003)
19	Temperature	Juvenile	4.37, NA	Commercially	(Fry & Hart, 1948)
20	Temperature	NA	NA,4.5	Commercially	(Hoyland et al., 1979)
21	Temperature	Embryo	NA	NA	(Urushibata et al., 2019)
22	Temperature	Larvae	0.00079,0.527	Commercially	(Motta et al., 2023)
23	Temperature	Embryo	NA	Commercially	(Wiegand et al., 1988)
24	Temperature	Embryo	0.1355,NA	Wild	(Battle, 1940)
25	Temperature	Adult (3-4	50,NA	Commercially	(Gillet et al., 1977)
		years)			
26	Oxygen	NA	4.26, NA	Commercially	(Dhillon et al., 2013)
27	Oxygen	NA	305.6, NA	Commercially	(van Ginneken et al., 1996)
28	Oxygen	Adult (3	100,NA	NA	(van den Thillart et al., 1976)
		years)			
29	Oxygen	Juvenile	6.08, 5.44	Commercially	(Fu et al., 2011)
30	Oxygen	NA	2.87,NA	Commercially	(Regan & Richards, 2017)
31	Oxygen	Juvenile	8.7, NA	Commercially	(Zhang et al., 2012)
32	Oxygen	Adult	15.62,9.54	Commercially	(Thoral et al., 2022)
33	Oxygen	NA	NA	Lab	(Kutty, 1968)
34	Oxygen	NA	104.5,16.24	NA	(Smit et al., 1971)

35	Oxygen	NA	25,NA	Commercially	(Bera et al., 2017)
36	Oxygen	NA	NA	Wild	(McNeil & Closs, 2007)
37	Ion	NA	22.7,NA	Commercially	(Schofield et al., 2006)
38	Ion	NA	38.15, 11	Commercially	(Wang et al., 2023)
39	Ion	Juvenile	107.5,21	Commercially	(Lawson & Alake, 2010)
40	Ion	NA	5.53,5.53	Commercially	(da Silva et al., 2021)
41	Ion	NA	8.99,8.15	Commercially	(Küçük, 2013)
42	Ion	NA	20.06, NA	Commercially	(Luz et al., 2008)
43	Ion	Juvenile	2.2, 5.4	Commercially	(Altinokand & Grizzle, 2001)
		(<6			
		months)			
44	Ion	Juvenile	10.07,8.4	Commercially	(Canagaratnam, 1959)
45	Ion	Embryo	NA	Lab	(Kong et al., 2013)
46	Ion	NA	12.18, NA	Commercially	(Moyson et al., 2016)
47	Ion	Larvae/Fry	0.0283,NA	NA	(Murai & Andrews, 1977)

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# Linking statement

Goldfish are often released in urban ponds and thus encounter potentially extreme temperature, oxygen, and chloride levels. In Chapter 1, I reviewed quantitative data on how goldfish respond to these abiotic variables. This review demonstrated the broad tolerance of the species and found that acclimation can improve performance under stressful conditions, possibly contributing to their establishment success in a range of habitats. This work also identified gaps in goldfish performance with respect to the effects of life stages and body size, and—most notably—in response to multiple interacting stressors.

In Chapter 2, I investigate the influence of warming and chloride pollution on the feeding performance of goldfish. Using a multifactorial experimental design, I measured the functional response of goldfish acclimated to a combination of warming and salinization levels that realistically reflect urban pond conditions.

# CHAPTER 2 - EFFECTS OF TEMPERATURE AND CHLORIDE SALINITY ON THE FUNCTIONAL RESPONSE OF GOLDFISH (*CARASSIUS AURATUS*)

# Abstract

Freshwater animals released into temperate urban environments are increasingly subjected to the effects of elevated water temperatures via climate warming and salinization via road salt application. In laboratory experiments, we tested the effects of these stressors on the feeding performance of goldfish (*Carassius auratus*), a globally invasive fish frequently released into urban ponds. Using goldfish from a pet supplier, we measured their functional response (prey consumption rate as a function of prey density) under combinations of two thermal treatments (18°C and 26°C) and two chloride treatments (0ppt and 0.96ppt). These treatments represent current and projected surface water temperatures and observed mean chloride contamination in ponds in the Montreal (Quebec) region. Feeding was maximal under the treatment combination that was nearest to the known thermal and iso-osmotic optima for goldfish (i.e., 26°C and 0.96 ppt chloride, respectively)—consistent with the environmental matching hypothesis, which posits that per capita effects, including foraging efficiency, increases as ambient conditions approach the physiological optimum. Experiments also revealed an interactive effect between temperature and chloride, suggesting that goldfish feeding varies with combinations of thermal and salinity conditions typical of urban ponds in temperate regions

Introduction

Urbanization facilitates the success and spread of introduced species, as urban centers are foci for transportation vectors (Borden & Flory, 2021; Marchetti et al., 2006; Ricciardi et al., 2017; Santana Marques et al., 2020) and anthropogenic stressors that can select and acclimate individuals to colonize other human-altered habitats (Anthropogenic Induced Adaptation to Invade Hypothesis: Hufbauer et al., 2012). In north-temperate latitudes in North America, warming and salt pollution are the prevailing stressors plaguing urban ponds. In urban regions susceptible to snow and ice, the application of road salt (sodium chloride, NaCl) causes chloride pollution in inland waters, with high inputs in the spring as snow melt and runoff are promoted by urban infrastructure and aesthetics (impervious surfaces) (Dugan et al., 2020). While peak chloride contamination occurs in spring, chloride ions are conservative and thus long-term application can cause chloride to accumulate and remain present in summer when ponds are simultaneously subjected to heating events (Sutherland et al., 2018). Urban heat island effects amplify the warming of urban ponds, which are typically shallow, lack rooted vegetation, and possess artificial bottom substrate (Brans et al., 2018).

Temperature and salinity are among the most influential environmental variables, directly affecting fish metabolism, oxygen consumption, survival, growth, and reproduction (Bœuf & Payan, 2001). Salinity plays a role in freshwater fish metabolism through the energetic costs of ionic and osmotic regulation for which growth is sacrificed (Altinokand & Grizzle, 2001). At salinities near a fish's iso-osmotic point—whereby osmotic equilibrium is established between the individual's body and its surrounding environment—fish should spend less energy regulating ions, allowing energy allocation to other tasks such as feeding (Walker et al., 2020).

Similarly, temperature can mediate the performance and distribution of introduced fishes by affecting metabolic rates (Richter et al., 2023). With warming waters, there is an increase in fish metabolic demands, requiring higher ingestion rates and possibly enhancing invader impact (Volkoff & Rønnestad, 2020). Temperature shifts toward an invader's thermal optimum are predicted to amplify its per capita effects (e.g., feeding rates) by promoting peak metabolic performance (The Environmental Matching Hypothesis: Iacarella et al., 2015; Ricciardi et al., 2013).

Efficiency in acquiring limiting resources, notably food, is a determining factor affecting colonization success and the impact of introduced nonnative species (Cuthbert et al., 2019). This

efficiency can be compared by quantifying the functional response—the relationship between prey consumption and prey availability (Holling, 1959)-across trophically similar species and environmental contexts using a standardized experimental protocol (e.g., Avlijaš et al., 2022; Cuthbert et al., 2019; Laverty et al., 2017; Mofu et al., 2019). Characteristics of the functional response curve provide information on the consumer's effect on a prey population. A linear (Type I) response is exclusively characteristic of filter feeding animals (Jeschke et al., 2004). A hyperbolic (Type II) response is considered destabilizing to a prey population owing to a lack of refuge from predation at low prey densities, whereas a sigmoidal (Type III) response is considered stabilizing owing to a reduced predation rate at lower prey densities (Avlijaš et al., 2022; Dick et al., 2013; Oaten & Murdoch, 1975). Key parameters of the response are attack rate (a, the rate of successful prey capture) and handling time (h, the time spent finding and feeding on prey); the *maximum feeding rate* is estimated as 1/h and is the asymptote of the response curve. These parameters allow for comparing responses and their inferred consequences for prey populations; however, in some instances, they can result in conflicting inferences, e.g. a low attack rate coupled with a high handling time. Synthesizing the key parameters in a single metric, the functional response ratio (FRR= a/h), has been proposed to have predictive value for invader risk assessment (Cuthbert et al., 2019). Specifically, the FRR can be useful for comparing impacts between species, populations, and contexts-including various abiotic stressors which can mediate the impact of an invader (Ricciardi et al., 2013).

A globally invasive species of emerging concern is the common goldfish (*Carassius auratus*), native to central China but now one of the world's most widely distributed aquatic invasive species. Despite being present in North America for centuries, recent reports of increased spread and local abundance (Boston et al., 2024) raise questions about the factors mediating goldfish invasiveness. Its widespread establishment can be credited to a broad physiological tolerance (see Chapter 1) (e.g. Ford & Beitinger, 2005; Schofield et al., 2006), but their local abundance and impacts are expected to be mediated by abiotic variables. Goldfish are commonly released as ornamental fish or as unwanted pets, and therefore are commonly found in urban waterways (Copp et al., 2005), which, in north temperate regions, are subjected to the acute stressors of warming and chloride salinity (from use of road salts). When the combined effects of temperature and salinity were investigated in goldfish, no interaction between these stressors was detected, but growth, food conversion and mean weight increased with salinity (0

vs 6ppt) and temperature (23°C vs 27°C), until critical conditions were reached (12ppt, 31°C) (Imanpoor et al., 2012). When investigated independently, salinities of 1-3ppt (Lawson & Alake, 2010; Luz et al., 2008) and temperatures in the range of 25-30°C (Chen et al., 2019; Nadermann et al., 2019; Pang et al., 2011) stimulate ingestion rates in non-larval life stages. Specific growth rates are generally optimal at temperatures in the range of 25-38°C (Audige, 1921; Imanpoor et al., 2012; Kestemont, 1995; Khieokhajonkhet et al., 2023) and salinities ≤1ppt (Altinokand & Grizzle, 2001; Imanpoor et al., 2012; Lawson & Alake, 2010). However, some literature reports optimal salinities of 6ppt and even higher (Küçük, 2013; Luz et al., 2008) (see Chapter 1). The influence of warming and salinity has specifically never been studied in the context of functional response for goldfish.

Using realistic temperature and salinity conditions in the laboratory, I tested the hypothesis that warming and salinization will individually mediate the feeding efficiency of goldfish. Specifically, I predicted that the functional response of goldfish will be higher at 26°C than at 18°C, owing to increased metabolic demands at higher water temperatures (Volkoff & Rønnestad, 2020). The elevated temperature corresponds with the reported thermal optimum of the species at non-larval life stages (Audige, 1921; Ferreira et al., 2014) and thus feeding performance should be enhanced at this temperature, according to the Environmental Matching Hypothesis (Iacarella et al. 2015). Similarly, I predicted that performance should be higher at 0.96 ppt of chloride, near the reported optimal isosmotic concentration (Altinokand & Grizzle, 2001; Lawson & Alake, 2010; Luz et al., 2008).

### Methods

### Animal provenance and acclimation

I used cultivated (pet store) goldfish as they are likely the source of urban invasions and thus can be model species to reveal traits enabling successful establishment in disturbed ecosystems (Copp et al., 2005). Goldfish (n= 61, 90.43mm  $\pm$  7.82 mm) were purchased from a pet supplier in Montreal, QC. The purchased fish were sized-matched using standard length (SL) to the approximate average length of an urban invasive population (caught in October 2022 at Beaver Pond, Montreal, QC, n=7, 123 mm  $\pm$  9.5mm) and to each other to account for variation in feeding rates (Rall et al., 2012). Clipping of the anal or caudal fin was used to individually identify fish; a method reported not to affect fish fitness (Dietrich & Cunjak, 2006; Radcliffe,

1950). Goldfish were housed in temperature-controlled chambers. Upon arrival from the pet store, fish were left to acclimate in a chamber at  $18^{\circ}$ C for a minimum of 24 hours, to allow water and chamber temperatures to equalize and avoid temperature shock. Fish were housed in 20-gallon (76 L) mesh-covered aquaria ( $50.8 \times 27 \times 31.12$  cm), with three fish per tank equipped with water filters and air stones following the McGill University animal care protocol (CCAC SOP 519 and AUP 8267). Each tank was stocked with vegetation mimics and coarse gravel substrate for habitat enrichment to encourage natural foraging behaviour (Smith & Gray, 2011). The chambers were kept on a 12h:12h (L:D) photoperiod to facilitate goldfish diurnal behaviour (Millsopp & Laming, 2008; Spieler & Noeske, 1984). Water quality variables (pH, ammonia, nitrate, nitrite) were tested weekly; ~30% of the water volume was replaced weekly or more frequently when indicated by inadequate water chemistry levels.

Fish were acclimatized to laboratory conditions for a minimum of 2.5 weeks before acclimating them to chloride and temperature treatments. Individuals were fed a ration of ~1% body weight using Nutrafin max sinking protein pellets (9 pellets/fish) on alternating days (Du et al., 2006). After the initial 2.5-week lab acclimation period, the subset of fish (n=31) intended for the salt treatment were acclimated to 0.96 ppt Cl. Pollution levels caused by road salt application can be measured in the form of chloride concentration (Dugan & Arnott, 2022) so I used chloride-specific treatments in my experiments. Tank chloride levels were monitored weekly using a YSI meter and Hach 2751340 Chloride Test Strips High Range, and these levels were adjusted as necessary for changes due to evaporation. The treatment level of 0.96ppt was chosen based on summer measurements of specific conductance of Montreal urban ponds converted to chloride values (Lévesque et al., 2020; Wallace & Biastoch, 2016). I used the highest recorded conductivity in Montreal ponds (1788  $\pm$  513  $\mu$ S/cm) to set the treatment threshold, which represents the current maximum possible exposure to chloride ions in a region where there is a known established urban population of goldfish (Beaver Pond Mont-Royal, Montreal, QC - from personal observations). Once chloride levels were reached, fish were acclimated to the treatment combination for four weeks prior to the functional response trials at 18°C. Once all trials were completed, a daily increase of 1°C occurred in the chamber until 26°C was established, upon which fish were left to acclimatize for four weeks to standardize temperature acclimation time among treatments (Grigaltchik et al., 2012; Sandblom et al., 2014). The same set of foraging trials were conducted at 26°C on fish that remained at their original chloride treatment. Fish were

reused both within and between temperature treatments, however never for the same resource density. This was accounted for in the statistical models (see statistical methods and results).

### Comparative functional response (FR) experiments

Fish were placed as individuals in 10-gallon aquaria  $(50.8 \times 19.05 \times 25.4 \text{ cm})$  equipped with an aerator and one plant mimic for habitat enrichment and filled with dechlorinated water and salt to reach housing treatment levels. The lateral sides of each tank were covered with black poster paper to minimize external visual stimuli and control for differences in goldfish consumption caused by shoaling behaviour (Murray et al., 2013; Roy, 1992). After a 24-hour starvation and acclimation period (Avlijaš et al., 2022; Mofu et al., 2019), each fish was fed a randomized density of commercialized food protein pellets. Other studies have found that cyprinids readily consume 1-2 mm pellets and are therefore useful in foraging experiments (Dominguez Almela et al., 2021; Mofu et al., 2019; Oyugi et al., 2012). Pellets were distributed equally across the tanks in 7 replicates of 8 densities (2, 5, 10, 15, 25, 50, 75, and 100 pellets), yielding 56 FR trials per treatment (224 trials in total) (Table S2.6). Fish were left undisturbed to feed for 1 hour (Guo et al., 2017). At the end of the FR trial, individuals were weighed and measured for standard length, returned to their respective holding tanks, and the remaining food items were counted (Mofu et al., 2019; Reid & Ricciardi, 2022). A control trial (i.e., with food pellets but no fish predator) was run at each density to confirm no pellet loss as a result of tank factors. Due to housing limitations, some fish were re-used after being permitted to rest for 7 days, but not for trials of the same resource density (Avlijaš et al., 2022; Reid & Ricciardi, 2022).

### Statistical Analyses

FR data were analyzed using R and R Studio (versions 4.2.2 and 2022.07.2, respectively) along with the *FRAIR* package, following Pritchard et al. (2017). A general workflow of model selection, model fitting, and model comparison was applied (Pritchard et al., 2017) using a combination of methods that allowed me to draw a confident consensus regarding the model (Avlijaš et al., 2022). Model selection is accomplished via three different methods. Firstly, a model is selected using Juliano's method which describes the shape of the response based on polynomial logistic functions. This method provides evidence for Type II or III functional

responses with the *frair\_test* function of the FRAIR package (Pritchard et al., 2017) and was chosen based on the non-replacement experimental design. The second model selection is done by fitting a scaling exponent (q) (such that, in Type II, q=0 and in Type III, q>0) using the *frair\_fit* function (Pritchard et al., 2017). Finally, I visually inspected the plotted curves displaying the relationship between the proportion of prey consumed versus prey provided, where a Type II response is indicated by a decreasing curve and a Type III response by an increase and then subsequently a decline (Avlijaš et al., 2022). When all three methods did not provide a unanimous consensus regarding curve type, the curve was described by the majority determination (Avlijaš et al., 2022).

When the model was chosen, it was then fitted using the *frair\_fit* function (Pritchard et al., 2017), which employs nonlinear model estimation optimized by using the maximum likelihood estimation incorporated in the FRAIR package (Pritchard et al., 2017). Type II responses were fit using Roger's random predator equation, and Type III were fit with the Hassel equation (Avlijaš et al., 2022; Pritchard et al., 2017). Here, models can be compared using information criteria, such as AIC, as a null hypothesis test is used. From these models, attack rate (*a*) and handling time (*h*) were extracted, using the Hollings Type II response from the basic equation, where N is prey density:

$$Consumption \ rate = \frac{aN}{1 + ahN}$$

Lastly, the model is compared among treatment groups. Since FR treatment curves were found to be the same type (see Results), fitted models were compared using *frair\_compare* which applies a z-test for likelihood ratio tests. In addition, 95% confidence intervals (CI) were produced for the models by bootstrapping the model fit and parameter estimates of the model (attack rate and handling time) (*frair\_boot*, n=999) (Iacarella et al., 2015; Pritchard et al., 2017; South et al., 2019). When CI did not overlap, it was deemed that there was no statistical difference (South et al., 2019).

To test for an interaction between temperature and chloride salinity on feeding, a generalized linear model (GLM) of bootstrapped estimates of FR parameters (n=30), using *frair\_boot* function and the *lme4* package (Bates et al., 2014) was used. For the GLM, data were fitted using a quasi-Poisson error distribution to analyze the effect of bootstrapped FR parameters (*a*, *h*, FRR and MFR) with respect to treatment interaction (chloride \* temperature). Interaction plots were

also used to visualize the magnitude of the interactions if detected using the package *ggplot2* (Wickham, 2016). Type III ANOVA was used to extract the effect size of the explanatory variable on the response variables using the *stats* package (Team, 2022).

Linear models and linear mixed models with z-scored numeric predictor variables were created using the *lme4* and *lmerTest* package and REML estimates (Bates et al., 2014; Kuznetsova et al., 2017) to test for the effects of treatments and other possible confounds. We used z-score transformed data to unify the scales of variables for comparison and correct for collinearity in the model. Linear Mixed Models (LMM) were used to analyze the effect of prey consumption with respect to temperature treatment, chloride treatment, the interaction of these treatments, and the possible interaction of fish length with the treatments (South et al., 2019). To create the models, random variables were tested for correlation and effect on residual variance. The random effects which I examined included the number of times each fish was used, both in total and for each specific treatment, as well as the experimental tank, housing tanks, fish ID, and the effect of variation in chloride acclimation time nested within different temperature conditions. Multicollinearity was accounted for by testing the correlation among predictor variables mass and length (method = "Pearson"). This method was also used to test for a correlation between fish size and density assigned, to investigate possible bias, following the detection of significance in the models. Models were compared with both AIC and BIC, again using the stats package (Team, 2022).

All model assumptions and auto-correlations were tested before statistical analysis. Change in fish mass after the duration of the experiments across all treatments was tested for significance using the Kruskal-Wallis test to account for the non-parametric data with the package *rstatix* (Kassambara, 2021).

# Results

Goldfish displayed Type II responses exclusively (Figure 2.1), confirmed by all three methods (Table 2.1, Figure S2.1, Tables S2.2 & S2.3). Control trials resulted in no pellet loss, indicating that changes in resources were due to predator consumption. According to the functional response ratio (FRR), the per capita effect of goldfish was maximal at the high-temperature/chloride combination (26°C/0.96ppt) and minimal at the low-temperature/chloride combination (18°C/0.96ppt) (Table 2.1, Figure 2.2). Generally, the FRR ratio was driven by

50

temperature; whereby fish under 26°C had higher FRR compared to 18°C, however, the effects of chloride varied between temperatures (Table 2.1, Figure 2.2). With respect to maximum feeding rate, fish showed a higher impact at high temperatures, however, the effect of chloride varied. Fish had a higher MFR at the high temperature/freshwater (26°C/0ppt) and the lowest at the low temperature/freshwater (18°C/0ppt) (Table 2.1, Figure 2.2).

Detailed investigation of model estimates a and h provided further insight into the variation in FRR and MFR impact results. The model estimates for a were all significantly different (p < 0.05), except for  $26^{\circ}$ C/0ppt compared to  $18^{\circ}$ C/0ppt or  $18^{\circ}$ C/0.96ppt (Figure 2.2, Table 2.2). The attack rate was higher at the high temperature when compared across the same chloride regime  $(26^{\circ}C/0.96ppt > 18^{\circ}C/0.96ppt)$ . The attack rate was higher for the no chloride treatment when compared to the same low-temperature treatment ( $18^{\circ}C/0.96ppt < 18^{\circ}C/0ppt$ ) (Table 2.2). The model estimates for *h*, were all significantly different (p < 0.05) among all treatments (Figure 2.2, Table 2.2). Higher temperature consistently corresponded with lower handling time, irrespective of chloride level (18°C/0ppt > 26°C/0ppt, 18°C/0.96ppt > 26°C/0.96ppt). When temperature treatments were compared across chloride regimes, opposite trends were detected: larger handling time without chloride at  $18^{\circ}C$  ( $18^{\circ}C/0ppt > 18^{\circ}C/0.96ppt$ ) and larger handling time with chloride at 26°C (26°C/0ppt < 26°C/0.96ppt) (Figure 2.1, 2.2). When data were bootstrapped and plotted (N=999), the 95% confidence intervals overlapped for all curves at high density, except for the no-salt treatments at  $26^{\circ}$ C and  $18^{\circ}$ C ( $26^{\circ}$ C >  $18^{\circ}$ C) (Table S2.4). At low resource densities, consumption was similar across all treatments indicated by the start of the FR curves (Figure 2.1).

No significant increase in fish length or mass was detected during the experiment (Figure 2.3). Fish length (standard length, mm) and mass were correlated (corr = 0.91), so only length was used as a measure of body condition in the model as it was the metric used to size-match fish. As a covariate, fish length affected consumption (as shown by the LMM; Table 2.4, Table S2.1), but was not correlated with the resource density assigned, which would have otherwise produced a bias (Figure S2.2, r (220) = -0.06, p = 0.37). The number of times an individual fish was reused both within the treatment and among treatments did not explain residual variance in the models and thus was not a random effect impacting consumption, owing to the minimum 7-day resting period (Figure S2.3). Chloride acclimation was tested for its effect nested among the temperature treatments as fish exposure to chloride was not independent of temperature. The

difference in acclimation time to chloride produced by the reuse of fish at  $18^{\circ}$ C and then  $26^{\circ}$ C did not significantly contribute to residual variance; its impact was accounted for in the chloride variable due to its high correlation (corr = 0.90). Individual fish ID did produce significant variation and accounts for variation explained by the housing and experimental tanks and was therefore included in models as a random effect. The results of the LMM reveal that while length affected the proportion of pellets consumed, temperature and chloride, nor their interaction, had a significant impact (Table 2.4).

Using bootstrapped estimates (N=30) of FR parameters, the generalized linear model result showed a significant interaction between temperature and chloride on attack rate and, less prominently, on handling time (Table 2.3; Figure 2.4). At 26°C, chloride increased the attack rate and handling time but had the opposite effect at 18°C. The FRR values reflect these changes in parameters as at 26°C the FRR values are larger and relatively balanced at both chloride levels, with the 0.96ppt treatment causing a slight increase. At 18°C, FRR values are lower overall, and the 0.96ppt treatment causes a decrease in FRR.

### Discussion

#### *Effect of temperature*

Our experimental measurements of the FRR suggest that goldfish will have a greater trophic impact at elevated temperatures near 26°C. Freshwater temperature groups (18°C/0ppt vs 26°C/0ppt) allow for a comparison of the sole effects of warming. As predicted, at 26°C the FRR and maximum feeding rate were higher and handling time was lower, but there was no difference in attack rate. The heightened performance at temperatures closer to the reported growth optimum of goldfish (i.e. 25-28°C; Audige, 1921; Ferreira et al., 2014) is consistent with the Environmental Matching Hypothesis and with the metabolic theory of ecology (Iacarella et al., 2015; Volkoff & Rønnestad, 2020; Walker et al., 2020).

Similar patterns have been reported for other FR studies at elevated and optimal temperatures: fish had higher maximum feeding rates, lower handling times, and lower attack rates, although in this study the difference in attack rate was not significant (Mofu et al., 2019). The inverse relationship between temperature and handling time has been explained in other studies by an increase in metabolic rate that allowed the predator to process more food before complete satiation (Robertson & Hammill, 2021). However, traits apart from temperature, such

as predator size, can influence the direction of this relationship (Buba et al., 2022), where lower handling times typically correspond with larger predators as larger sizes incur greater metabolic demands in ectotherms (Buba et al., 2022). From our LMM results, individual fish size did influence the amount consumed; however, there was no significant difference in fish size between treatments, indicating that fish size does not explain variation in parameters across temperatures.

The general relationship between temperature and attack rate has been shown to be hump-shaped (unimodal), such that at lower or higher temperatures there is a decline in food search and capture efficiencies (Buba et al., 2022; Englund et al., 2011; Uiterwaal & DeLong, 2020). As such, handling time and attack rate can be maximized at different temperatures (Uiterwaal & DeLong, 2020). In our case, although 26°C corresponds with one reported aquaculture growth optimum for goldfish, the optimum for the particular individuals used here may be at an intermediate temperature or towards the high range of their reported optima (Buba et al., 2022), which could explain why there were no significant differences in attack rate along the range of 18-26°C.

In another FR study, it was found that goldfish had high attack rates and low handling time (thus higher maximum feeding rate) at 13°C (Dickey et al., 2022). Similarly, although salt diminishes the FRR of goldfish at 18°C in our study, the lower handling time at 18°C/0.96ppt caused higher maximum feeding rates (Figure 2.2).

#### *Effect of chloride*

When solely comparing the effects of chloride under the high-temperature regime (26°C: 0ppt vs 0.96ppt), we find support for the Environmental Matching Hypothesis: overall impacts measured via FRR are highest when both variables are at the reported growth optima (26°C and 0.96ppt). We predicted this to be driven by a reduction in handling time; instead, at 26°C, handling time significantly increased with salt and no difference in attack rate was noted. The reduced efficiency in consuming and digesting pellets with salt exposure is reflected in the lower maximum feeding rate at the 26°C/0.96ppt treatment. While it has been reported that goldfish food conversion rate increases with salinity, this occurs at salinities higher than in this study and in fish of smaller size (Altinokand & Grizzle, 2001; Luz et al., 2008). Luz et al. (2018) found that goldfish are significantly more efficient at digesting food at salinities up to 2ppt and at 18°C,

which matches well with our finding of a significantly lower handling time and higher MFR at 18°C/0.96ppt treatment combination; however, FRR is reduced in the salinity treatment (18°C/0ppt vs 18°C/0.96ppt), contrary to our second prediction. At low temperatures, we expected to see the highest impact with chloride, which is only observed among the individual FR parameters: higher maximum feeding rate and lower handling time at 18°C. The overall impact is likely reduced at low temperatures, as the addition of chloride significantly reduced attack rates. Reduced attack rates at low salinity and 18°C were also observed in another study that examined temperature and salinity effects on the FR of a freshwater amphipod (Cuthbert & Briski, 2021).

### Temperature and chloride interaction

We found a complex interaction between temperature and chloride salinity, such that the effect of chloride has opposing effects at high resource density (handling time) and low resource density (attack rate), depending on temperature. The FRR suggests that salinization at realistic chloride concentrations will dampen per capita effects at 18°C but will amplify them at 26°C. This has implications for the timing of goldfish release into urban waterbodies. During spring when runoff increases chloride pollution, the release of cultivated goldfish will have a lower overall trophic impact (FRR) than if they are released during the summer when the inert chloride ions have accumulated. However, if the goldfish are established and persist until the next spring, impacts are likely to increase.

The results of the linear mixed model (LMM), whereby only length influenced the proportion of food items consumed, underscore the importance of considering individual variation in per capita effects. While generalized GLM analysis focuses on the interaction of stressors on FR parameters *a* and *h*, the LMM emphasized that individual fish traits, such as size, can be crucial in determining resource consumption. For example, goldfish are often released into ponds when they outgrow their home aquaria, among other reasons such as illness, aggressive behaviour and rapid reproduction (Gertzen et al., 2008). Therefore, the size structure of an invading population would be useful data for post-establishment risk assessment.

### Functional response caveats

While we detected Type II curves for all treatments, the experimental design might be biased to produce this relationship owing to a lack of complexity and non-mobile prey. Despite using protein pellets to accord with the carnivorous aspect of the goldfish diet, our results can only truly be extrapolated to the detritivore aspect of their omnivorous diet. In another study that conducted FR trials using both frozen chironomid and live *Gammarus pulex* as prey (Guo et al., 2017), the authors found a Type II response for both prey items, but attack rate and handling time were lower with the live prey. In addition, both our study and Guo et al. (2017) found that the per capita effect of goldfish is influenced by body size, as detected in model comparisons. The incorporation of body size, along with other data—such as shoaling and foraging behaviours, and local abundance—into predictive models would be more informative. For example, some FR metrics (e.g. Dickey et al., 2020) incorporate a relative abundance of non-native consumers and trophically analogous natives, thereby integrating classical functional response with a proxy for numerical response.

### Implications for risk assessment and management

We tested aquarium-reared individuals of goldfish as they are the most likely source of invasion into urban ponds through pet release (Copp et al., 2005). In urban environments, resource inputs from runoff can be higher, and chloride pollution and climate warming effects are amplified (Brans et al., 2018; Pagliaro & Knouft, 2020). Thus, urban ponds could select for traits that confer goldfish with enhanced invasiveness under rapid environmental change (cf.Hufbauer et al., 2012). If urban ponds act as acclimation hubs, the spread of urbanized goldfish into natural waterbodies could add competitive pressure on native benthic fishes as environmental conditions shift toward those in urban landscapes. The number of individuals that could be acclimated and subsequently distributed from urban areas could be enormous, given that thousands of goldfish purchased from urban centers like Montreal are estimated to be released annually (Gertzen et al., 2008). Overall, these results suggest that pet-store goldfish have the potential to promote trophic impacts when released into urban environments, such that the greatest per capita effects of goldfish may occur at water quality conditions near the species' growth optimum, but these impacts vary with combinations of multiple environmental stressors. Finally, urban ponds can facilitate the acclimation of introduced fish species to warming more

than native species in natural environments, while also accommodating invaders that are preadapted to warmer climates (Correa & Gross, 2008; Marchetti et al., 2006).

# **Tables and Figures**

**Table 2.1.** Results of four methods for determining best fit (Type II or III) for the functional response at each treatment. Parameter estimates for each functional response treatment were derived with the *frair\_fit* and *frair\_compare* functions where *a*=attack rate, *h*=handling time. All models fitted can be found in Table S2.1 of Supplementary Information.

Treatment	Friar_test	1 <sup>st</sup>	$q \pm SE, p$	Lowest	Visual	Fit	a ± SE,	h ± SE,	Max	FRR
		order		AIC	Inspection	Used	Р	Р	feeding	(a/h)
		term, p		value					rate	
									(1/h)	
18°C – 0ppt	Type II	-0.03, 2.2e-16 ***	0.8 ± NA, NA	Type III	Type II	Type II	6.5 ± 1.2, 1.438e- 08 ***	0.07 ± 0.003, 2.2e- 16 ***	13.7	89.8
18°C - 0.96ppt	Type II	-0.03, 2.2e-16 ***	-0.4± 0.3, 0.18232	Type III	Type II	Type II	3.4 ± 0.5, 5.994e- 11 ***	0.06 ± 0.003, 2.2e- 16 ***	16.9	56.9
26°C – 0ppt	Type II	-0.03, 2.2e-16 ***	-0.9± NA, NA	Type III	Type II	Type II	4.8 ± 0.6, 1.497e- 13 ***	0.04 ± 0.002, 2.2e- 16 ***	23.6	112.7
26°C - 0.96ppt	Type II	-0.03, 2.2e-16 ***	0.2± 0.3, 0.52085	Type II	Type II	Type II	6 ± 0.9, 1.105e- 10 ***	0.05± 0.002, 2.2e- 16 ***	20.1	119.2

Curve classified using the Juliano method in which the direction of the 1<sup>st</sup> order term (+or -) indicates type II or III curve respectively. The hypothesis testing method indicates type III if q>0. Model fitting is done by fitting q=0 or allowing q to vary. Models are compared with AIC. Asterisk indicate significant results (\*p<0.1, \*\*p<0.01, \*\*\*p<0.001) and we report Standard Error (SE). Visual inspection (Figure S1.1) of pellets provided vs. proportion of pellets eaten also allows us to classify the FR curve type. Maximum feeding rate and functional response ratio were calculated with all decimals and rounded here to one decimal.

**Table 2.2.** P values for parameter comparisons (a, attack rate and h, handling time) for the<br/>functional response of pet store goldfish at each treatment. Parameters are compared using the<br/>friar\_compare function. Significance is denoted with "\*" and we report the standard error.18°C -0ppt18°C -0.96ppt26°C -0.96ppt

	18°C -0ppt	18°C -0.96ppt	26°C -0ppt	26°C -0.96ppt
18°C-0ppt	-	a: 0.012* ± 1.27	a: 0.18 ± 1.32	a: 0.015* ±1.06
		h: $0.0044* \pm$	h: 2e-16 *** ±	h: $0.019* \pm 0.004$
		0.0048	0.0039	
18°C-0.96ppt	-	-	a: $0.09 \pm 0.83$	a: 0.015* ± 1.06
			h: 2e-05*** ±	h: $0.02* \pm 0.004$
			0.004	
26°C-0ppt	-	-	-	a: 0.3 ± 1.13
				h: 0.013* ±0.003
26°C-0.96ppt	-	-	-	-

Sig: '\*\*\*' 0.001, '\*\*' 0.01, '\*' 0.05

**Table 2.3.** Results from GLM with a quasi-Poisson error distribution were used to determine differences in bootstrapped (N=30) estimates of attack rate and handling times with regards to chloride and temperature treatments and their interaction, using a Type III Anova and  $\chi^2$  to report the effect size of our explanatory variables on the response variable.

a) Attack rates

Factor	Chisq	Df	p-value
Chloride	0.8842	1	0.347013
Temperature	0	1	0.995349
Chloride x Temperature	10.8609	1	0.001082 *

# b) Handling times

Factor	Chisq	Df	p-value
Chloride	6.571	1	0.01036 *
Temperature	274.353	1	<2.2e-16 ***
Chloride x Temperature	51.893	1	5.861e-13 ***

## c) Results for a/h ratio

Factor	Chisq	Df	p-value
Chloride	0.4042	1	0.52494
Temperature	2.3632	1	0.12422
Chloride x Temperature	8.5901	1	0.00338 **

Asterisks denote significant p values (. < 1; \* < 0.05; \*\*\* < 0.001)

Error 0.023 0.023	217 217	26.81	<2e-16***
0.023 0.023	217 217	26.81 1.41	<2e-16***
0.023	217	1.41	0.16
0.023	217	-0.18	0.86
0.023	217	-0.30	0.76
0.024	217	3.31	0.00108**
	0.023 0.024	0.0232170.024217	0.023217-0.300.0242173.31

Tab	le 2.4	<b>.</b> R	Results from	the L	MM b	est fi	t Linea	ar re	egres	ssion mode	els a	issessin	g the e	ffects of
accli	imati	on	treatments (	tempe	erature	x ch	loride)	on	the	proportion	of	pellets (	eaten.	
г.	1				Г.		<b>C</b> .	1	1	10		1		1

Sig: \*\*\*\* 0.001, \*\*\* 0.01, \*\* 0.05 Temperature: Chloride = interaction between chloride and temperature standardized using Z score



**Figure 2.1.** The functional response of goldfish under four different treatments. Pet-store goldfish (n=61) were fed one of 8 known densities using protein fish meal pellets (densities= 2,5,10,15,25,50,75,100), and 7 replicates were conducted at each density (n= 56 trials per treatment, Table S2.6). Fish were acclimated to the treatment conditions for 4 weeks. The shaded area represents 95% confidence intervals obtained by bootstrapping using the *frair\_boot* function (N=999), the line represents the fitted model, and the circles are the experimental observations.



**Figure 2.2.** Results and standard error (SE) for the pairwise comparisons among the core FR parameters a) attack rate and b) handling time for the functional response of pet store goldfish at each treatment. Parameters are compared using the friar\_compare function, and significance is denoted by different letters. The parameters were synthesized and plotted to show c) the functional response ratio and d) the maximum feeding rate of the goldfish under the four treatments. The SE was extracted from the bootstrapped (N=30) values for FRR and MFR using the frair\_boot function (N=999). The significance difference is not denoted as c) and d) are estimated and not observations.



**Figure 2.3.** Change in (a) length and (b) mass after the three-month experiment across the treatments. There is no significant difference between the groups (a) Kruskal-Wallis, P = 0.15, b) Kruskal-Wallis., P = 0.7. Circles are observations, and bars are standard errors.



**Figure 2.4.** Interaction plots for the mean values of the bootstrapped FR parameters a) attack rate, b) handling time and their synthesis c) Functional Response Ratio. The y-axis is the response variable for the interaction between the x-variable (chloride treatment, ppt) and the plotted line variable (temperature, °C). All plots show an interaction where all parameters depend on temperature and chloride (Table 2.3). The bootstrap coefficient (N=30) of the parameters using *friar\_boot* was used to create GLM with quasi-Poisson error distribution.

# Supplementary Material

**Table S2.1.** Results from linear models and linear mixed models with Z score variables tested for correlation with the proportion of pellets (response variable), including random effects that produced significant residual variance. Fish length and mass are very correlated, so only length was used as fish were sized matched to this measurement. The interaction terms was always included in the model to investigate their effects. Best fitting model based on AIC and BIC is bolded.

Model	(intercept)	Prop	Temp*Cl	L	Temp:L	Cl:L	(1 ID)	df	AIC	BIC
1	0.623991	+	+				+	6	200.1771	220.5932
2	0.623975	+	+	+			+	7	197.0780	220.8967
3	0.626110	+	+	+	+		+	8	204.1002	231.3216
4	0.6230	+	+	+		+	+	8	216.6875	243.9089
5	0.6286	+	+		+	+	+	8	223.7986	251.0201
6	0.626547	+	+		+		+	7	206.9203	230.7391
7	0.6261	+	+			+	+	7	217.0156	240.8343

\*(1|ID) = the random effect of individual fish, CL.Acc = Acclimation time to chloride treatment, L= Fish standard length (mm) standardized using Z score, Cl = Chloride treatment standardized using Z score, Temp = Temperature treatment standardized using Z score, prop= proportion of pellets consumed, Temp:L = interaction between temperature and fish length standardized using Z score, Temp\*Cl = interaction between chloride and fish length standardized using Z score,

models are	001404			
Model	18°C-0ppt	26°C-0ppt	18°C-0.96ppt	26°C-0.96ppt
Flex (III)	350.7767	348.9908	335.5610	292.1474
Type III	350.9286	360.9998	338.0839	291.9739
TYPE II	356.8066	353.2930	336.0746	290.5335

 Table S2.2. AICc scores comparing model fits for each of the 4 treatments. The best-fitting models are bolded.

Treatment	Fit	Response	$a\pm$ stand. err., p	h± stand. err., p
18°C-0ppt	Flex (type III)	"flexpnr"	n/a	n/a
18°C-0ppt	(type III) type III	"hassIIInr"	n/a	0.0790 ±0.0039, 6e-16
18°C-0ppt	type II	"rogersII"	6.55 ±1.16, 1.46e-08	0.0729 ±0.003, 2.2 e-16
26°C-0ppt	Flex (type III)	"flexpnr"	n/a	n/a
26°C-0ppt	type III	"hassIIInr"	n/a	4.58e-02±1.57e-0, 2.2e-16
26°C-0ppt	Type II	"rogersII"	4.78±0.646,1.51e-1 3	0.042±0.0020,2.2e -16
18°C- 0.96ppt	Flex (type III)	"flexpnr"	n/a	0.0490± 0.0123,6. 90e-05
18°C- 0.96ppt	type III	"hassIIInr"	n/a	5.95e-02± 2.58e-0 3,< 2.2e-16
18°C- 0.96ppt	type II	"rogersII"	3.37 ± 0.515, 5.99e -11	0.0593 ± 0.0034,< 2.2e-16
26°C-0ppt	Flex (type III)	"flexpnr"	n/a	$0.0515 \pm 0.0032,$ < 2e-16
26°C-0ppt	type III	"hassIIInr"	n/a	0.0510± 0.0027, < 2e-16
26°C-0ppt	type II	"rogersII"	5.94± 0.921, 1.10e- 10	0.0499± 0.0023, < 2.2e-16

 Table S2.3. Estimated results using frair\_fit

\*\*n/a indicates that there is no fit

Treatment	Attack rate (a)	Handling time ( <i>h</i> )	Fit used
18°C-0ppt	3.914 - 19.344	0.059 - 0.088	type II
26°C-0ppt	3.103 - 9.311	0.033 - 0.053	type II
18°C-0.96ppt	2.151 - 6.377	0.046 - 0.074	type II
26°C-0.96ppt	3.708 - 15.319	0.043 - 0.06	type II

 Table S2.4. Bootstrapped 95% confidence intervals of parameter estimates using frair\_boot.

 Since bootstrap is less than 100 used we do not record Bca. We report percentile CI

**Table S2.5.** Summary of average housing conditions and standard deviation  $(\pm)$  for the treatment group during the 6.5-week housing period and the trial conditions (2.5 weeks lab acclimation + 4 weeks treatment acclimation). Water parameters were checked weekly using YSI, thermometer and API water chemistry testing kits.

Treatment	Housing	Housing	Experimental	Experimental
	<b>Temperature</b> (C)	Chloride (ppt)	<b>Temperature</b> (C)	Chloride (ppt)
$18^{\circ}C - 0ppt$	$18.02\pm0.24$		$18.37\pm0.3$	
$18^{\circ}\mathrm{C} - 0.96\mathrm{ppt}$	$18.02\pm0.24$	$0.945\pm0.022$	$18.37\pm0.3$	$0.958 \pm 0$
$26^{\circ}C - 0ppt$	$25.76\pm0.33$		$25.7\pm0.69$	
$26^{\circ}C - 0.96ppt$	$25.76\pm0.33$	$0.920\pm0.037$	$25.7\pm0.69$	$0.958 \pm 0$

**Table S2.6.** Summary of FR replicates treatment and pellets density (sinking food pellets) for goldfish (*Carassius auratus*) populations (total of 224 trials). One fish died from the 26°C/0.96ppt treatment group, explaining the difference in sample size and df. but all other fish remained visibly healthy during the experimental trials.

Treatment	2 pellets	5 pellets	10 pellets	15 pellets	25 pellets	50 pellets	75 pellets	100 pellets	Total Trials
18°C-0ppt	7 replicates	56							
18°C- 0.96ppt	7 replicates	56							
26°C-0ppt	7 replicates	56							
26°C- 0.96ppt	6 replicates	7 replicates	55						



**Figure S2.1.** Visual inspection to diagnose FR type by plotting the relationship between the proportion of pellets consumed and pellets provided for all four treatments. The line is a smooth curve fitted by loess using the geom\_smooth function from the ggplot package in R. All curves indicate Type II as the curves generally have continuous declining slopes.



**Figure S2.2**. Pelley density assigned to each fish's standard length in the four treatments (corr = -0.06, method = Pearson)


**Figure S2.3.** Residual variance of times an individual fish was reused a) per treatment and b) in total,c) individual fish, d)the different chloride acclimation time nested in temperature treatments, e) experimental tank and f) holding tanks (both e. and f. are accounted for in c. fish ID). Only individual fish have a large variation.

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# Linking statement

In Chapter 2, I measured goldfish functional response under a conservative chloride concentration and realistic temperatures. The highest per capita effects occurred for either variable when they more closely matched the respective growth optima for temperature and salinity. However, the experiments also revealed an interaction between chloride and temperature stressors on the functional response. In Chapter 3, using physiological metrics, I investigated how the combination of warming and chloride stressors influence the thermal tolerance of goldfish, using critical thermal maxima methods. For this study, I added an intermediate temperature (21°C) and used a higher chloride level (6ppt), to test how thermal performance varies after acclimation and exposure to values deviating from the reported optima for temperature and salinity. These added treatment levels are ecologically relevant thresholds, preserving the integrity of the management applications of my results.

# CHAPTER 3 - EFFECTS OF ACCLIMATION TO TEMPERATURE AND CHLORIDE SALINITY ON THE THERMAL TOLERANCE OF GOLDFISH (*CARASSIUS AURATUS*)

# Abstract

The critical thermal maximum (CTmax) of cultivated goldfish (*Carassius auratus*) obtained through the pet trade was measured to assess how their thermal tolerance is affected by acclimation to realistic levels of abiotic stressors. Goldfish were exposed to a combination of chloride concentrations selected based on those caused by road salt pollution (0ppt, 1ppt, 6ppt) and elevated temperature based on climate projections specific to the Great Lakes basin (18°C, 21°C, 25°C). Results revealed a linear response to acclimation temperatures, with those fish exposed to temperatures near the published species' optimum having the highest CTmax, irrespective of chloride treatment. Thermal tolerance further characterized using metrics of agitation and acclimation potential revealed that high chloride levels (6ppt) can cause sub-optimal performance during heat stress, but acclimation to intermediate temperatures buffers the negative effects. The effects of multiple stressors on thermal tolerance could mediate the invasive potential of pet-store goldfish released in urbanized contexts. Goldfish populations presently acclimating to heat and salt stress in urban ponds would likely have a competitive advantage when subsequently introduced to wild ponds that are altered by these stressors under expanding urbanization in future years.

## Introduction

Temperature determines the distribution of species, including fish, by governing metabolic rates: the speed at which resources are obtained and converted to usable energy (Hutchison, 1961). The direct impact of temperature on physiology and behaviour renders data from thermal metrics such as critical thermal maxima and minima (CTmax and CTmin) essential prerequisites for effective conservation management of ectotherms under climate change (Teal et al., 2018). CTmax measures an organism's lethal upper thermal tolerance and can test how thermal tolerance is affected by acclimation history (Becker & Genoway, 1979; Stillman, 2003). In CTmax experimental trials, fish acclimated to a certain temperature will be subjected to a constant rate of increase in temperature until their locomotor activity becomes disorganized with continued exposure (Bennett & Beitinger, 1997). Temperature and time are recorded upon detecting a defined response—typically the *loss of equilibrium* (LOE), which, in fish, is indicated when the animal tips over or assumes an atypical belly-up position (Christensen et al., 2020); at this point, the fish has become so disoriented that it loses the ability to escape conditions that would impact its fitness or result in death (Becker & Genoway, 1979; Bennett & Beitinger, 1997; Wells et al., 2016).

Agitation temperature ( $T_{ag}$ ) is recorded when a fish searches for cooler water refuge, exhibited as rapid swimming and interpreted as avoidance behaviour (Kochhann et al., 2021; McDonnell & Chapman, 2015; Wells et al., 2016). Thermal tolerance capabilities are further revealed by measuring the *agitation window* ( $T_{aw}$ ), the difference between CTmax and the agitation temperature (Wells et al., 2016), and comparing it to the LOE. A large difference between  $T_{aw}$  and LOE indicates suboptimal tolerance capacities, such that the fish cannot maintain normal behaviour for long periods during heating events (Kochhann et al., 2021), thereby impacting fitness.

Data on these thermal tolerance metrics are of particular urgency for informing risk assessment, as the greatest effect of climate change on species is not increasing annual means but rather increasing extreme temperatures (Stachowicz et al., 2002). In inland waters of north temperate regions, increasing salinity is a co-occurring stressor with climate warming (Jeppesen et al., 2020); both salinity and temperature directly affect fish metabolism, oxygen consumption, survival, growth, and reproduction (Bœuf & Payan, 2001). Urban environmental traits (e.g. dark colours, impervious material, reduced vegetation buffer) result in amplified warming due to the absorption of energy—the urban "heat island effect" (Touchaei & Wang, 2015; Wang & Akbari, 2016). Urban ponds experience higher mean and maximum summer temperatures with pronounced daily fluctuations (Brans et al., 2018). Wild (rural and semi-rural) ponds and lakes are predicted to experience similar conditions as they become increasingly altered by expanding urbanization. Mean maximum summer surface water temperatures in the lower Great Lakes are projected to shift from circa 18°C to 26°C (Trumpickas et al., 2009), perhaps driven further by increasing urbanization (Howard & Gerber, 2018). Expansion of road density and associated impervious surfaces increases the demand for road salt application and facilitates runoff of chloride contaminants in melting snow during spring thaws. Sodium chloride (NaCl) is the predominant deicer used in northeastern North America, causing chloride pollution to increase in parallel with urbanization (Dugan & Arnott, 2022; Dugan et al., 2017; Dugan et al., 2020). In Canadian urban ponds, chloride levels range from 1ppt (Montreal, QC; Lévesque et al., 2020; Wallace & Biastoch, 2016) to 6ppt (Col. S. Smith Reservoir, Toronto, Canada; Mayer et al., 1999).

In addition to warming and chloride pollution, a third plague of urban areas is invasive species. Understanding an invader's response to these abiotic stressors is critical to understanding their invasion success and potential ecological impacts. A globally invasive fish commonly released as unwanted pets, goldfish (*Carassius auratus*), have become widespread and locally abundant in urban areas (Copp et al., 2005). Broad environmental tolerance has contributed to rendering this ornamental fish both popular in the pet trade and successful in becoming established in new environments. Goldfish can tolerate temperatures up to 44.7°C (Ferreira et al., 2014) and salinities up to 30ppt (Wang et al., 2023). Reported optimal temperature levels for growth are generally in the range of 25–28°C (Audige, 1921; Imanpoor et al., 2012; Kestemont, 1995; Khieokhajonkhet et al., 2023). Reported optimal salinity levels for growth are generally 0-1ppt (Altinokand & Grizzle, 2001; Imanpoor et al., 2012; Lawson & Alake, 2010), some studies report 6ppt or higher (Küçük, 2013; Luz et al., 2008) (see Chapter 1).

The theory of osmoregulation predicts that increases in salinity towards the iso-osmotic point require the species to expend less energy for ionic regulation as internal osmolarity is closer in match to the ambient environment (Walker et al., 2020). In addition, the metabolic theory predicts that warming increases an animal's metabolic rate (Walker et al., 2020). At optimal temperatures, a heightened metabolic rate can optimize growth and reproduction. Urban

environmental conditions are warming urban waters (Brans et al., 2018; Hester & Bauman, 2013) possibly aligning with the growth optimum for goldfish (~26°C). This overlap may occur in the Great Lakes as they are predicted to warm up to 26°C (Iacarella et al., 2015). Taken in conjunction with the environmental matching hypothesis, which posits that an environmental shift towards an invader's optimum increases per capita (Iacarella et al., 2015), both theories justify an examination of the limits of goldfish thermal tolerance and invasiveness under current and projected conditions to improve invasive species risk assessment.

Studies have investigated the influence of salinity on fish thermal tolerance with divergent results. In some cases, salinity significantly affected the thermal tolerance, with acclimation to salinities nearing the iso-osmotic optimum causing increased Ctmax (*Acipenser medirostris* :Sardella et al., 2008) or increased resistance to lethal temperature exposure (*Rhinomugil corsula*: Kutty et al., 1980). While salinity can improve tolerance through interactive effects, above a certain temperature the positive effects of salinity can be reversed (Haney & Walsh, 2003). Other studies have found that salinity does not impact thermal tolerance (Hines et al., 2019) or that the timing of salinity acclimation plays an important role (Shaughnessy & McCormick, 2018). These studies report on anadromous, eurythermal and euryhaline fishes where fluctuations in salinity and temperature are frequently encountered; whereas very few studies focus on stenohaline fishes (such as goldfish, sensu Lahlou et al., 1969) restricted to freshwater conditions. Both temperature and salinity impact goldfish performance (see Chapter 1) and are common co-occurring stressors in urban environments, but no studies have yet investigated how environmentally relevant salinity concentrations affect goldfish thermal tolerance.

To evaluate the effects of warming and salinity on the thermal tolerance of goldfish, I tested predictions informed by metabolic theory of ecology and iso-osmotic theory. Based on the hypotheses that the thermal tolerance of goldfish is dependent on acclimation to elevated temperature and salinity, and that the capacity to respond to stress is elevated within the species' optimum range (Walker et al., 2020), I predicted that thermal tolerance will be maximal with acclimation to temperatures and salinities nearest the optima reported for goldfish, i.e. 25°C and 0-1ppt (Audige, 1921; Ferreira et al., 2014; Luz et al., 2008). Furthermore, I predicted that thermal tolerance will be higher when one environmental variable (salinity or temperature) is

within the species' optimal range and the other is suboptimal than when both variables are suboptimal.

## Methods

#### Animal provenance and acclimation

Pet store goldfish were purchased from a pet store located in Montreal, Quebec. Fish with a mass of  $15.75g \pm 6.60$  and standard length of  $7.72cm \pm 0.55$  were ordered to represent juveniles that are likely age 1, based on published studies (Lorenzoni et al., 2007). Since no external features allow for identifying sex in goldfish, juveniles were chosen for CTmax trials as opposed to adults to control for possible effects of sex on thermal resistance (Hollands, 1956) and know salinity tolerance during that life stage (1-10ppt, <6ppt is stressful) (Lawson & Alake, 2010; Luz et al., 2008).

Goldfish were housed in climate-controlled chambers at the McGill phytotron. Similar housing and animal care conditions were maintained for the CT max trials as in previous FR trials (see Chapter 2), according to the McGill animal care protocol (SOP519 and AUP 8267). Fish tanks were populated with 2 fish per tank and fed  $\sim 0.5\%$  of their body weight daily using commercialized protein pellets (3 Nutrafin sinking pellets/day) (Du et al., 2006). Experiments were conducted with goldfish acclimated to temperatures of 18°C, 21°C and 25°C, which are within the ranges of the current and forecasted mean maximum summer surface water temperatures of the nearshore Great Lakes (Trumpickas et al., 2009, 2015). An intermediate value of 21°C was added to allow a more complete exploration of the environmental matching hypothesis by allowing the detection of any deviation from reported optimums. Acclimation to 0ppt, 1ppt and 6ppt salinity concentrations were used. These salinity treatments were chosen to mimic the highest chloride levels recorded in Montreal area ponds (Chapter 1; Lévesque et al., 2020; Wallace & Biastoch, 2016) and around a reported maximum chloride level of an urban waterbody in Toronto, Canada (Col. S. Smith Reservoir) receiving runoff from a multiway highway (~6ppt) (Mayer et al., 1999). They are also within goldfish iso-osmotic thresholds, not yet stressful for juveniles (Altinokand & Grizzle, 2001; Lawson & Alake, 2010; Luz et al., 2008). Salinities higher than 6ppt have been shown to negatively affect growth, food intake and conversion, increase cortisol and cause muscle dehydration (Luz et al., 2008).

Following a 2.5-week acclimation period to lab conditions, fish were brought to their treatment temperature through a daily change of 1°C in the growth chamber until either 18°C, 21°C or 25°C was established. The chloride treatments were initiated in increments of 1ppt per day until the treatment level of either 1ppt or 6ppt was reached and timed so that temperature and chloride treatment levels achieved final levels on the same day. Other studies have done similar salinity increments with no adverse effects (Küçük, 2013). This was done by adding table salt (Windsor Brand, NaCl) and dissolving it in dechlorinated water. Salinity was monitored using YSI instrument, and chloride was recorded using Hach 2751340 Chloride Test Strips High Range. Fish were acclimated to their treatment conditions for 3 weeks, as done in other long-term acclimation trials (i.e., Bennett & Beitinger, 1997; McDonnell et al., 2021; Nyboer & Chapman, 2017; Reid & Ricciardi, 2022)

#### Critical thermal maxima (CTmax) experiments

Upper thermal tolerance limits (CTmax) of goldfish were tested following established protocols (i.e.,McDonnell & Chapman, 2015; McDonnell et al., 2021; Reid & Ricciardi, 2022; Wells et al., 2016). Following a 24-hour starvation period, one fish was transferred to a 10-gallon tank (50 x 19 x 25 cm), inside an individual isolation box (i.e., a commercial breeding box; 26 x 15 x16 cm), with opaque divers to prevent fish from seeing the other fish in the second experimental setup. The initial water conditions of the experimental aquaria were the same as the treatment conditions of the fish being tested. The fish was left to acclimate for 1 hour, 30 minutes in the breeding tank with aeration and 30 minutes with the heater [a temperature-control unit (A JULABO CORIO<sup>TM</sup> CD heating immersion circulator)] circulating to ensure acclimation to trial condition (Potts et al., 2021; Reid & Ricciardi, 2022).

Trials were initiated by increasing water temperature at a fixed rate of 0.3°C/min (Becker & Genoway, 1979). Two trials were run simultaneously and recorded using a camera. The recording was revisited to verify observations post-trial. Exposure to an increase in temperature continued until fish displayed a loss of equilibrium (LOE), denoting the CTmax temperature (Becker & Genoway, 1979; Bennett & Beitinger, 1997; Hutchison, 1961). Here, I defined LOE as: a non-lethal endpoint marked when a fish fails to maintain dorsal-ventral orientation for a minimum of three seconds (Becker & Genoway, 1979; Bennett & Beitinger, 1979; Bennett & Beitinger, 1997; Hutchison, 1961). Another behaviour change, agitation, was noted throughout the trial (Kochhann et al.,

2021; McDonnell & Chapman, 2015). Agitation temperature (Tag) was defined when the fish began swimming around the tank quickly and agitatedly, lasting longer than 40 seconds with an attempt to escape the isolation box. This behaviour was determined based on pilot trials. Temperature and trial length were recorded using JULABO EasyTEMP Professional software. After the display of LOE, temperature increases were halted, and the fish was returned to a recovery tank equipped with 30°C aerated water, which was gradually cooled back to the respective treatment temperature. After recovery, fish were weighted (g) and measured (standard length, mm). No fish was reused for another CTmax trial, and if a death occurred during the trial, the data was discarded. A total of 59 CTmax trials were conducted with 5-7 replicates performed for each treatment.

From the two behavioural observations (CTmax,  $T_{ag}$ ), additional metrics were derived: thermal agitation window ( $T_{aw}$ , the difference between CTmax and  $T_{ag}$ ; Wells et al., 2016), acclimation agitation window ( $A_{aw}$ , the difference between  $T_{ag}$  and acclimation temperature; McDonnell et al., 2021), and modified thermal safety margin (TSM, the difference between CTmax and the acclimation temperature; McDonnell et al., 2021).

#### Statistical Analysis

All data were analyzed and visualized using R and R Studio (version 4.2.2 and 2022.07.2). The effect of acclimation temperatures and chloride on the response variables CTmax,  $T_{ag}$ ,  $T_{aw}$ ,  $A_{aw}$  and TSM were tested for goldfish using linear mixed models with treatment and condition factor as a fixed effect and housing tank as a random effect using the *lme4* package (Bates et al., 2014). The condition factor was calculated using the Fulton factor (K = mass/length<sup>3</sup>) with a modified equation (K= mass/ (standard length<sup>0.29</sup>100), where the exponent was derived from the regression of log (mass) against log (length) (Richter et al., 2000). The confirmation of fixed effects included in the model for each response variable was supported by AIC and BIC scores using the *stats* package (Team, 2022). The random effect of housing tanks was included in each model to account for potential variability among individuals due to housing conditions. Based on the results, a linear regression including temperature and chloride, its interaction, as a fixed effect was selected for all 5 response variables. Type III ANOVA was used to test for differences in response variables CTmax,  $T_{ag}$ ,  $T_{aw}$ ,  $A_{aw}$ , and TSM as a result of the explanatory variables using the *car* package (Fox & Weisberg, 2019). The effect size was

calculated using Eta partial squares. To generate pairwise estimates, post hoc Tukey-Kramer tests were conducted with 95% confidence intervals. All model assumptions were investigated and validated using Levene's test of equal variance, and the Shapiro-Wilk test for normality and plotting residuals using the *car* package. Pairwise comparisons between response variables (CTmax and Tag) were made using t-test. All data was visualized by plotting it using *ggplot2* package (Wickham, 2016).

## Results

CTmax was observed to vary in response to acclimation temperature and chloride independently, but not by the interaction. Goldfish displayed significantly higher CTmax values when acclimated to 21°C and 25°C, compared to 18°C (Figure 3.1; Tables 3.1 & 3.2), with CTmax increasingly linearly with acclimation to increasing temperatures. Chloride treatment of 1ppt and 6ppt also contributed to higher CTmax, although to a lesser extent than temperatures 25°C and 21°C (Table 3.1 & 3.2). For chloride, there was no linear relationship between acclimation and thermal capabilities; 1ppt induced the longest time display before LOE, followed by 6ppt then freshwater conditions (0ppt). Since no interaction was detected, our results do not support the prediction that acclimation to a combination of optimal variables (25°C/0 or 1ppt) would exhibit optimal response. Instead, we found that acclimation to optimal temperature (25°C) and chloride (1ppt) independently promote heightened response, with temperature having a larger effect. Freshwater conditions (0ppt) did not significantly promote enhanced thermal response. The effect of temperature was even stronger at 21°C, below the reported optimum, in comparison to the effects of chloride treatments (1ppt and 6ppt) (Table 3.1).

The thermal agitation temperature ( $T_{ag}$ ) was influenced by temperature and chloride, independently, as well as by their interaction (21°C/6ppt) (Table 3.1 & 3.2). High temperature (25°C) acclimation resulted in a higher thermal agitation temperature, whereas high chloride treatment (6ppt) caused a decline; however, the interaction of 21°C/6ppt buffered the effects of high chloride by causing a significantly higher  $T_{ag}$  value (Figure 3.2; Table 3.2). For each of the nine treatments, there was a significant difference between the temperature where fish displayed LOE (CTmax) and their agitation temperature (Table S 2.2). As such, the thermal agitation window ( $T_{aw}$ ) follows a similar trend whereby chloride levels of 6ppt reduced thermal capacities but, the response was buffered at intermediate temperatures through a significant interaction at 21°C/6ppt (Figure 3.2; Table 3.2). However, temperature alone did not influence  $T_{aw}$  (Table 3.1 & 3.2). The acclimation agitation window (A<sub>aw</sub>) was also influenced in the same manner; negatively affected by 6ppt chloride and positively by the interaction of 21°C/6ppt, with temperature alone having no impact (Figure 3.3, Table 3.1). Conversely, the thermal safety margin is influenced by temperature acclimation to 18°C, and by 6ppt chloride, but to the latter a small degree (Table 3.1). For all metrics analyzed, the fish condition factor (Fulton's index) did not explain variation in the response variable, as indicated through the AIC and BIC results of the models (Table S3.1). Only one fish died during the CTmax trials under the treatment of 18°C/1ppt.

## Discussion

Both temperature and chloride exposure individually mediated the CTmax of goldfish, with temperature having a stronger effect. The results did not support the prediction that acclimation to multiple optimal conditions would have additive effects on thermal tolerance. Instead, they suggested that intermediate temperatures buffer the negative effects of high chloride exposure on the agitation and thermal windows of goldfish.

CTmax experiments highlighted the broad thermal capabilities of goldfish, which were able to withstand temperatures up to 40.04°C before displaying LOE. This aligns with reported upper thermal tolerances of goldfish acclimated to a similar temperature (Ferreira et al., 2014; Ford & Beitinger, 2005; Khieokhajonkhet et al., 2023; Yanar et al., 2019). Here, as in other studies, there is a uniformly positive trend between thermal acclimation and CTmax, which holds across a broad range of body masses, 2.45–16.47g (Khieokhajonkhet et al., 2023; Yanar et al., 2023; Yanar et al., 2019). Salinity tolerance has been found to vary with goldfish life stage (and thus body size), with tolerance of younger fish reduced (max tolerance of 6ppt: Imanpoor et al., 2012) compared with older life stages (max tolerance of 20ppt; Küçük, 2013). In this study, we did not find an effect of size (mass and length, via Fulton's condition factor) on CTmax, contrary to what was reported for other species (Recsetar et al., 2012). We investigated the effects of chloride on the tolerance of a single stage (juveniles), whereas comparisons with other life stages would have allowed an assessment of ontogenetic patterns.

We predicted an enhanced capacity for goldfish to respond to acute thermal stress when acclimated to multiple conditions within its optimum range. There was no interaction detected for the 25°C/1ppt or 25°C/0ppt treatments, indicating a lack of support for our prediction. Instead, we found acclimation at the optimal temperature of 25°C yielded the highest CTmax results (40.40°C) irrespective of chloride, followed by acclimation at the temperature of 21°C. The optimal salinity level of 1ppt contributed to a heightened CTmax response, as did the salinity treatment of 6ppt, but this was not the case for the freshwater condition. The lack of thermal enhancement in freshwater suggests that the salinity optimum for thermal tolerance capacity is closer to 1-6ppt (Küçük, 2013; Luz et al., 2008).

Acclimation to temperatures of <21°C and salinities of <6ppt likely reduce the energy needed for metabolism and osmoregulation, allowing for an enhanced response to acute heat stress (as revealed by higher CTmax values). While this study assumed a growth optimum for salinity near 1ppt, there is much inconsistency in the literature concerning reported tolerance and performance across life stages and fish size (see Chapter 1), whereby the reported optimum is much higher in some cases (Imanpoor et al., 2012; Küçük, 2013), which perhaps explains why we detected positive effects at 6ppt.

It was also predicted that acclimation to either one of the variables at an optimal range would heighten thermal tolerance when the other variable was non-stressful. While there was support for this claim at the 25°C treatments, enhanced thermal capabilities were not consistent at the 1ppt or 0ppt treatment. These results might reflect a potential trade-off in energy allocation for goldfish, whereby the cost of metabolic regulation is more reduced than that of osmoregulation near the optimal temperature. At these treatment levels, temperature appears to be a primary driver, and chloride is secondary, in mediating the physiological capabilities of goldfish (Walker et al., 2020).

Conversely, acclimation temperature alone played no influence on the thermal agitation window; instead, salinities of 6ppt accelerated the onset agitation behaviour in comparison to the other reference groups, creating a larger thermal window. However, acclimation to 25°C affected the agitation temperature itself. During acute heat stress episodes, the conditioned avoidance response of goldfish is sensitive to acute temperature changes (cf. Hoyland et al. 1979), with heightened avoidance behaviour occurring in 25°C acclimated fish. While 6ppt prolongs the onset of LOE, possibly through a reduction in iso-osmotic regulation cost, salinity-associated behavioural changes may be at play by reducing T<sub>ag</sub> and thus increasing T<sub>aw</sub>. Another study, by Lawson and Alake (2010), found that non-stressful salinities (4–10 ppt) have been associated

with weaker threat response via increases in erratic movement. Their results help explain our findings, which showed that, though acclimation to temperatures (21–25°C) allows goldfish to withstand greater heat stress, acclimation to salinity 6ppt increases avoidance behaviour, thereby causing sub-optimal performance under heat stress conditions. At a sub-optimal temperature of 21°C, the onset of avoidance behaviour is delayed in comparison. Reynolds and Casterlin (1979) found an 'activity well' (a decline in activity) in goldfish with acclimation to a temperature near their final preferendum (28°C). We hypothesize that, when combined with another stressor, this 'activity well' shifts owing to a trade-off in energy allocation of metabolism and osmoregulation. The high chloride level also impedes the ability of goldfish to acclimate to temperature changes, as indicated by the acclimation agitation window; however, there is an overlap between when the fish begin to agitate and their acclimation window at the 6ppt treatment and the intermediate temperature of 21°C.

Some studies suggest that thermal optima for aquatic ectotherms are overestimated in the absence of consideration of metabolic demands and energy acquisition (Buba et al., 2022; Delong et al., 2018; Uiterwaal & DeLong, 2020). In contrast to a presumed monotonic relationship between temperature and metabolic rate, fish might instead perform optimally at an intermediate temperature in a unimodal response (Delong et al., 2018; Uiterwaal & DeLong, 2020). In our study, the interaction of temperature and chloride stressors could change optimal levels for each variable through a trade-off in energy costs (cf. Walker et al., 2020). For other stenohaline fishes, stress response was strengthened with chronic exposure to high salinities strengthened, but was weakened with temperature increases; presumably, high salinities reduce the baseline concentration of cortisol, which in turn facilitates stress reactivity when exposed to additional stressors (Walker et al., 2020).

#### Implications for goldfish invasion risk

These results reveal the resilience of goldfish to two major stressors encountered in urban watersheds. Acclimation to high temperature and salinity increases CTmax, but consideration of other thermal tolerance metrics ( $T_{ag}$ ,  $T_{aw}$ ,  $A_{aw}$ , TSM) shows that high salinities have negative effects on thermal tolerance that are buffered at intermediate temperatures. These results suggest acute responses to increases in temperature, such as in the form of heat waves that are expected to increase in frequency and intensity in aquatic ecosystems (Woolway et al., 2021).

The realistic treatment scenarios used here add insight to the role of urban ponds in acclimating goldfish to anthropogenic stressors. Chronic exposure to high temperatures and salt pollution in urban ponds could confer a competitive advantage to goldfish as they subsequently invade wild (rural and semi-rural) lentic ecosystems that will likely become increasingly urbanized in the future. This is analogous to the scenario in which populations adapted to human-altered habitats in a native range perform well when invading similar human-altered habitats in a novel range ('Anthropogenically Induced Adaptation to Invade' hypothesis; Hufbauer et al., 2012).

Seasonal changes and timing of salt spike events can also influence species establishment. The capacity for goldfish to acclimate to urban stressors is compromised via a narrow acclimation window at 6ppt salinities, diluting their potential to tolerate heat stress events—such as heat waves or general warming trends. The early onset of agitation behaviour that generally occurs at 6ppt can also hinder goldfish establishment or post-establishment success, as a consequence of fish replacing routine adaptive behaviour (such as foraging, shelter refuge, and feeding) with thermal avoidance behaviour (Wells et al., 2016). Given that this redirection of energy could result in reduced competitiveness if natives are more tolerant to chloride, multi-stressor experiments should be conducted on various resident species commonly found in habitats invaded by goldfish. Tables and Figures

Fixed effects	Estimate	Standard Error	df	t-value	p-value
		Critical Thermal	Maximum (CTm	ax)	_
(Intercept)	35.66	0.34	48	105.52	<2e-16***
21 °C	2.02	0.43	48	4.68	1.34e-05***
25 °C	4.39	0.44	48	9.91	3.40e-13***
1ppt	1.08	0.44	48	2.44	0.019*
6ppt	0.94	0.44	48	2.12	0.039*
21 °C:1ppt	0.18	0.60	48	0.31	0.76
25 °C:1ppt	-0.89	0.63	48	-1.43	0.16
21 °C:6ppt	-0.77	0.60	48	-1.29	0.20
25 °C:6ppt	-0.59	0.61	48	-0.96	0.34
		Agitation Te	mperature (T <sub>ag</sub> )		
(Intercept)	30.05	0.89	45	33.83	<2e-16***
21 °C	1.44	1.16	45	1.24	0.22
25 °C	5.21	1.16	45	4.48	5.01e-05***
1ppt	1.83	1.20	45	1.52	0.14
бррt	-7.60	1.16	45	-6.54	4.97e-08***
21°C:1ppt	0.36	1.67	45	0.22	0.83
25 °C:1ppt	-2.39	1.67	45	-1.43	0.16
21 °C:6ppt	5.86	1.60	45	3.65	0.00068***
25 °C:6ppt	1.70	1.60	45	1.06	0.30
<u></u>	1110	Thermal Agita	tion Window (Ta	,)	0.00
(Intercept)	5.61	0.95	45	5 91	4.24e-07***
21 °C	0.65	1 24	45	0.52	0.61
25°C	-0.83	1 24	45	-0.67	0.51
1ppt	-1.08	1.29	45	-0.84	0.40
6ppt	8 54	1 24	45	6.87	1.61e-08***
21 °C:1ppt	-0.033	1.79	45	-0.018	0.99
25 °C:1ppt	1.83	1.79	45	1.03	0.31
21 °C:6ppt	-6.70	1.72	45	-3.90	0.00031***
25 °C:6ppt	-2.28	1.72	45	-1.33	0.19
<u></u>	2.20	Acclimation Agit	tation Window (A	(aw)	0.12
(Intercept)	12.05	0.89	45	13.56	<2e-16***
21 °C	-2.56	1.16	45	-2.20	0.033*
25 °C	-1.79	1.16	45	-1.54	0.13
1ppt	1.83	1 20	45	1.52	0.13
6ppt	-7.60	1 16	45	-6 54	4.97e-08***
21 °C·1ppt	0.36	1.67	45	0.22	0.83
25 °C:1ppt	-2.39	1.67	45	-1 43	0.05
21 °C:6ppt	5.86	1.60	45	3 65	0.00068***
25 °C · 6nnt	1 70	1.60	45	1.06	0.30
<u></u>	1., 0	Thermal Safe	tv Margin (TSM)	1.00	0.00
(Intercent)	17.66	0.31	45	56 51	e-16***</td
$21^{\circ}C$	_1.00	0.41	45	-4 68	2.63e-05***
$21^{\circ}$ C	-1.52	0.41	45	-6 39	8 236-08***
25 C	0.74	0.42		1 76	0.086
Thh	0.74	0.42	40	1.70	0.000

**Table 3.1**. Linear regression models assessing the effects of acclimation treatments (temperature x chloride) on thermal tolerance metrics of *C. auratus*

бррt	0.94	0.41	45	2.29	0.027*	
21 °C:1ppt	0.33	0.59	45	0.56	0.58	
25 °C:1ppt	-0.56	0.59	45	-0.95	0.35	
21 °C:6ppt	-0.84	0.56	45	-1.49	0.14	
25 °C:6ppt	-0.59	0.56	45	-1.04	0.31	

Asterisks denote significant p values (\*\*\* < 0.001; \*\* < 0.01; \* < 0.05)

calculated using Eta partial squares. Models used were based in the best in AIC efferna.								
Factor	Chisq	Df	<i>p</i> -value	Effect size				
Critical Thermal Maximum (CTmax)								
(Intercept)	11135.06	1	<2e-16***	1				
Acclimation Temperature	100.66	2	<2e-16***	0.94				
Acclimation Chloride	6.68	2	0.036*	0.51				
Interaction (Temperature*Chloride)	6.30	4	0.178	0.50				
Agitatio	n Temperatu	re (T <sub>ag</sub> )						
(Intercept)	1144.370	1	2.2e-16***	0.98				
Acclimation Temperature	23	2	1.015e-05***	0.98				
Acclimation Chloride	82.47	2	<2.2e-16***	0.98				
Interaction (Temperature*Chloride)	20.83	4	0.00034***	0.98				
Agitation Window (T <sub>aw</sub> )								
(Intercept)	34.93	1	3.42e-09***	0.61				
Acclimation Temperature	1.70	2	0.43	0.61				
Acclimation Chloride	79.76	2	<2.2e-16***	0.61				
Interaction (Temperature*Chloride)	22.35	4	0.00017***	0.61				
Acclimation A	Agitation Win	dow (AA	<b>W</b> )					
(Intercept)	183.98	1	<2.2e-16***	0.89				
Acclimation Temperature	4.95	2	0.084	0.89				
Acclimation Chloride	82.47	2	<2.2e-16***	0.89				
Interaction (Temperature*Chloride)	20.83	4	0.00034***	0.89				
Thermal Safety Margin (TSM)								
(Intercept)	3192.93	1	<2.2e-16***	1				
Acclimation Temperature	42.21	2	6.83e-10***	1				
Acclimation Chloride	5.57	2	0.062	1				
Interaction (Temperature*Chloride)	6.42	4	0.17	1				

**Table 3.2**. Results of a Type III ANOVA examining the effects of acclimation treatments (temperature x chloride) on the thermal tolerance metrics of *C. auratus*. Effect size was calculated using Eta partial squares. Models used were based in the best fit AIC criteria.

Asterisks denote significant p values (\*\*\* < 0.001; \*\* < 0.01; \* < 0.05)



**Figure 3.1**. Mean values of critical thermal maximum (CTmax;  $\pm$ SE) of goldfish (*Carassius auratus*) to the 9 possible treatments, grouped here by the three acclimation temperatures plotted by their chloride level. Fish were acclimated to treatments for 3 weeks before trials (n=54). Post hoc Tukey Kramer test (95% CI) reveals significant differences between the temperature groups and chloride groups, but not between chloride levels within the temperature groups.



**Figure 3.2.** Mean values of critical thermal maximum (CTmax;  $\pm$ SE) and thermal agitation temperature (T<sub>ag</sub>;  $\pm$ SE) of goldfish (*Carassius auratus*) acclimated to the 9 possible treatments, grouped here by the three acclimation temperatures plotted by their chloride level (n= 57). Fish were acclimated to treatments for 3 weeks before trials. The differences between CTMax and T<sub>ag</sub> were all statistically significant (t-test) and the distance represents the thermal acclimation window (Taw)



**Figure 3.3.** Mean values of thermal agitation window and thermal acclimation window ( $\pm$ SE) of goldfish (*Carassius auratus*) to the 9 possible treatments, grouped here by the three acclimation temperatures plotted by their chloride level (n= 57). Fish were acclimated to treatments for 3 weeks before trials.



**Figure 3.4**. Thermal safety margin (TSM;  $\pm$ SE) of goldfish (*Carassius auratus*) acclimated to the 9 possible treatments, grouped here by the three acclimation temperatures plotted by their chloride level (n= 57). Fish were acclimated to treatments for 3 weeks before trials.

# Supplementary Materials

Treatment	Housing Temperature (°C)	Housing Chloride (ppt)	Experimental Temperature (°C)	Experimental Chloride (ppt)
18 °C – 0ppt	$18.17\pm0.26$	$0\pm 0$	$18.2\pm0.49$	$0 \pm 0$
$18^{\circ}\mathrm{C}-1\mathrm{ppt}$	$18.17\pm0.26$	$947 \pm 15.56$	$17.87\pm0.49$	$1.02\pm0$
18 °C – 6ppt	$18.17\pm0.26$	$6021.5 \pm 204.35$	$17.86\pm0.27$	$5.90\pm0$
$21 ^{\circ}\mathrm{C} - 0 \mathrm{ppt}$	$20.95\pm0.54$	$0\pm 0$	$21.26\pm0.29$	$0\pm 0$
$21 ^{\mathrm{o}}\mathrm{C} - 1 \mathrm{ppt}$	$20.95\pm0.54$	$998 \pm 89.02$	$21.96 \pm 0.85$	$1.02 \pm 0$
$21 ^{\mathrm{o}}\mathrm{C}-6ppt$	$20.95\pm0.54$	$6117.17 \pm 219.86$	$22.08 \pm 0.64$	$5.90\pm0$
$25 \ ^{o}C - 0 ppt$	$24.75{\pm}0.53$	$0\pm 0$	$25.37\pm0.89$	$0\pm 0$
$25 \ ^{o}C - 1 ppt$	$24.75{\pm}0.53$	$1003.67 \pm 41.19$	$24.5\pm0.27$	$1.10 \pm 0$
$25 ^{\mathrm{o}}\mathrm{C}-6ppt$	$24.75{\pm}0.53$	$5863.17 \pm 452.41$	$24.64\pm0.34$	$5.90\pm0$

**Table S3.1.** Summary of average temperature and chloride during the fish-holding period (3 weeks) and experimental tanks ( $\pm$ SE). Housing measurements were recorded weekly and experimental measurements were recorded before initiating a trial.

**Table S3.2**. AIC and BIC scores of linear regression models and linear mixed model fits for goldfish critical thermal maximum (CTmax), agitation temperature (Tag), agitation window (Taw), acclimation agitation window (Aaw), and thermal safety margin (TSM) (e). The lowest AIC and BIC scores are bolded.

Model	Intercept	Temp *	(1 HT)	Condition	Condition:	Condition:	df	AIC	BIC
		Chloride			Cl	Temp:Cl			
CTmax									
1	34.82	+	+	+			12	160.28	184.79
2	35.66	+	+				11	147.84	170.31
3	34.86	+	+		+		14	186.55	215.15
4	33.36	+	+			+	20	254.13	294.99
Tag									
1	28.04	+	+	+			12	238.60	262.46
2	30.05	+	+				11	227.50	249.38
3	26.49	+	+		+		14	259.76	287.60
4	25.56	+	+			+	20	316.80	356.58
	Taw								
1	6.71	+	+	+			12	245.90	269.77
2	5.61	+	+				11	233.54	255.42
3	8.44	+	+		+		14	267.21	295.06
4	7.81	+	+			+	20	322.51	362.29
					Aaw				
1	10.04	+	+	+			12	238.60	262.46
2	12.05	+	+				11	227.50	249.38
3	8.49	+	+		+		14	259.76	287.60
4	7.56	+	+			+	20	316.80	356.58
					TSM				
1	16.76	+	+	+			12	145.43	169.30
2	17.66	+	+				11	133.51	155.39
3	16.92	+	+		+		14	171.92	199.77
4	15.36	+	+			+	20	241.75	281.53

Note: Temp \* Chloride = acclimation temperature and chloride interaction term plus their individual effects, HT = Housing tank, Condition = Fish Condition using Fulton's Factor, Condition:Cl = the interaction of fish condition and acclimation chloride, Condition:Cl:Temp= the interaction of fish condition and acclimation chloride and temperature

Treatment	Mean difference	t-value	p-value
18 °C -0ppt	5.61	10.47	0.00047***
18 °C -1ppt	4.53	3.84	0.012*
18 °C -6ppt	14.15	25.75	2.26e-07***
21 °C -0ppt	6.26	5.28	0.0019**
21 °C -1ppt	5.15	8.10	0.0013**
21 °C -6ppt	8.10	12.70	5.39e-05***
25 °C -0ppt	4.78	16.83	2.81e-06***
25 °C -1ppt	5.54	27.10	1.10e-05***
25 °C -6ppt	11.04	7.86	0.00053***

**Table S3.3.** Results of the paired students t-test comparing the CTmax and Tag for each of the nine respective treatments.

Asterisks denote significant p values (\*\*\* < 0.001; \*\* < 0.01; \* < 0.05)

value)           Critical Thermal Maximum (CTmax)           Temperature           18 °C vs 21 °C         2.02         0.43         NA         (4.68)         <1e-05***							
Critical Thermal Maximum (CTmax)           Temperature           18 °C vs 21 °C         2.02         0.43         NA         (4.68)         <1e-05***							
Temperature           18 °C vs 21 °C         2.02         0.43         NA         (4.68)         <1e-05***           18 °C vs 25 °C         4.39         0.44         NA         (9.91)         <1e-05***           21 °C vs 25 °C         2.37         0.39         NA         (6.05)         <1e-05***           0ppt vs 1ppt         1.08         0.44         NA         (2.44)         0.039*           0ppt vs 6ppt         0.94         0.44         NA         (2.12)         0.086           1ppt vs 6ppt         -0.14         0.40         NA         (-0.35)         0.94           Temperature (Tag)           Temperature           18 °C vs 21 °C         1.44         1.16         NA         1.23         0.43           18 °C vs 25 °C         5.22         1.16         NA         4.49         <0.001****							
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Oppt vs 1ppt         1.08         0.44         NA         (2.44)         0.039*           Oppt vs 6ppt         0.94         0.44         NA         (2.12)         0.086           1ppt vs 6ppt         -0.14         0.40         NA         (-0.35)         0.94           Agitation Temperature (Tag)           Temperature           18 °C vs 21 °C         1.44         1.16         NA         1.23         0.43           18 °C vs 25 °C         5.22         1.16         NA         4.49         <0.001***							
Oppt vs 6ppt         0.94         0.44         NA         (2.12)         0.086           1ppt vs 6ppt         -0.14         0.40         NA         (-0.35)         0.94           Agitation Temperature (Tag)           Temperature (Tag)           18 °C vs 21 °C         1.44         1.16         NA         1.23         0.43           18 °C vs 25 °C         5.22         1.16         NA         4.49         <0.001***							
Ippt vs 6ppt         -0.14         0.40         NA         (-0.35)         0.94           Agitation Temperature (Tag)         Temperature (Tag)           18 °C vs 21 °C         1.44         1.16         NA         1.23         0.43           18 °C vs 25 °C         5.22         1.16         NA         4.49         <0.001***							
Agitation Temperature (Tag)           Temperature           18 °C vs 21 °C         1.44         1.16         NA         1.23         0.43           18 °C vs 25 °C         5.22         1.16         NA         4.49         <0.001***							
Temperature           18 °C vs 21 °C         1.44         1.16         NA         1.23         0.43           18 °C vs 25 °C         5.22         1.16         NA         4.49         <0.001***							
18 °C vs 21 °C1.441.16NA1.230.4318 °C vs 25 °C5.221.16NA4.49<0.001***							
18 °C vs 25 °C 5.22 1.16 NA 4.49 < <b>0.001</b> ***							
<u>21 °C vs 25 °C</u> 3.78 1.06 NA 3.56 <b>0.0011**</b>							
Chloride							
Oppt vs 1ppt         1.83         1.20         NA         1.52         0.28							
Oppt vs 6ppt         -7.60         1.16         NA         -6.54         <1e-04***							
1ppt vs 6ppt         -9.43         1.11         NA         -8.53         <1e-04***							
Interactions							
18 °C -0ppt vs 18 °C -1ppt -1.83 1.22 21.8 -1.50 0.084							
18 °C -0ppt vs 18 °C -6ppt 0.68 1.21 18.3 0.67 1							
18°C -1ppt vs 18°C -6ppt       9.43       1.12       21.4       8.45       <0.001***							
21 °C -0ppt vs 21 °C -1ppt -2.19 1.17 19.8 -1.86 0.64							
21 °C -0ppt vs 21 °C -6ppt 1.74 1.12 21.4 1.56 0.81							
21°C -1ppt vs 21°C -6ppt 3.90 1.22 21.8 3.23 0.075							
25 °C -0ppt vs 25 °C -1ppt 0.567 1.17 19.8 0.483 1							
25 °C -0ppt vs 25 °C -6ppt 5.90 1.11 17.4 5.32 0.0013**							
25°C -1ppt vs 25°C -6ppt 5.34 1.21 18.3 4.41 0.0077**							
18°C -0ppt vs 21°C -0ppt -1.44 1.17 19.8 -1.23 0.94							
18°C -0ppt vs 25°C -0ppt -5.22 1.17 19.8 -4.45 0.0063**							
21°C -0ppt vs 25°C -0ppt -3.78 1.07 19.0 -3.53 <b>0.045</b> *							
18°C -1ppt vs 21°C -1ppt -1.80 1.22 21.8 -1.48 0.85							
18°C -1ppt vs 25°C -1ppt -2.82 1.22 21.8 -2.32 0.37							
21°C -1ppt vs 25°C -1ppt -1.03 1.27 20.3 -0.81 1							
18°C -oppt vs 21°C -oppt -/.29 1.12 21.4 -0.53 < <b>U.UUU1</b> ***							
18°C -oppt vs 25°C -oppt -6.91 1.11 1/.4 -6.24 <b>0.0002</b> ***							
$\frac{21 \circ \text{C-oppt Vs } 25 \circ \text{C-oppt}}{\text{Thermal A site for Window (TAW)}}$							
Tomperature							
$\frac{18^{\circ} \text{C vs} 21^{\circ} \text{C}}{18^{\circ} \text{C vs} 21^{\circ} \text{C}} = 0.65 = 1.24 \text{NA} = 0.52 = 0.86$							
$18^{\circ} \text{C vs} 25^{\circ} \text{C}$ $-0.83$ $1.24$ NA $-0.67$ $0.78$							
$21 ^{\circ}\text{C} \text{ vs} 25 ^{\circ}\text{C}$ $-1.48$ $1.14$ NA $-1.30$ $0.30$							
Chloride							
0ppt vs 1ppt -1.08 1.29 NA -0.84 0.68							
0ppt vs 6ppt 8.54 1.24 NA 6.87 <1e-04***							

**Table S3.4**. Results of post hoc Tukey-Kramer test with 95% confidence intervals examining the effects of acclimation treatment (temperature x chloride) on the thermal tolerance metrics for *C.auratus*. Only showing relevant pairwise comparisons

1ppt vs бppt	9.62	1.18	NA	8.14	<1e-04***		
Interactions							
18 °C -0ppt vs 18 °C -1ppt	1.08	1.30	21.8	0.83	1		
18°C -0ppt vs 18°C -6ppt	-8.54	1.25	19.8	-6.81	<0.0001***		
18°C -1ppt vs 18°C -6ppt	-9.62	1.19	21.4	-8.06	<0.001***		
21 °C -0ppt vs 21 °C -1ppt	1.12	1.25	19.8	0.89	0.99		
21 °C -0ppt vs 21 °C -6ppt	-1.84	1.19	21.4	-1.54	0.82		
21 °C -1ppt vs 21 °C -6ppt	-2.96	1.30	21.8	-2.27	0.40		
25 °C -0ppt vs 25 °C -1ppt	-0.75	1.25	19.8	-0.60	1		
25 °C -0ppt vs 25 °C -6ppt	-6.26	1.19	17.4	-5.28	0.0015**		
25 °C -1ppt vs 25 °C -6ppt	-5.50	1.29	18.3	-4.26	0.012*		
18 °C -0ppt vs 21 °C -0ppt	-0.65	1.25	19.8	-0.52	1		
18 °C -0ppt vs 25 °C -0ppt	0.83	1.25	19.8	0.66	1		
21 °C -0ppt vs 25 °C -0ppt	1.48	1.14	19.0	1/19	0.92		
18 °C -1ppt vs 21 °C -1ppt	-0.61	1.30	21.8	-0.47	1		
18 °C -1ppt vs 25 °C -1ppt	-1.00	1.30	21.8	-0.77	1		
21 °C -1ppt vs 25 °C -1ppt	-0.39	1.36	20.3	-0.29	1.00		
18°C -6ppt vs 21°C -6ppt	6.05	1.19	21.4	5.07	0.0013**		
18 °C -6ppt vs 25 °C -6ppt	3.11	1.19	17.4	2.63	0.24		
21 °C -6ppt vs 25 °C -6ppt	-2.94	1.23	19.5	-2.38	0.35		
	Acc	limation Agitat	ion Window				
		Temperati	ıre				
18 °C vs 21 °C	-2.56	1.16	NA	-2.20	0.070		
18 °C vs 25 °C	-1.79	1.16	NA	-1.53	0.27		
21 °C vs 25 °C	0.78	1.06	NA	0.73	0.74		
		Chloride	2				
Oppt vs 1ppt	1.83	1.20	NA	1.52	0.28		
Oppt vs oppt	-7.60	1.16	NA	-6.54	<1e04***		
1ppt vs 6ppt	-9.43	1.11	NA	-8.53	<1e-04***		
		Interactio	ns				
18°C -0ppt vs 18°C -1ppt	-1.83	1.22	21.8	-1.50	0.84		
$18^{\circ}\text{C}$ -0ppt vs $18^{\circ}\text{C}$ -6ppt	7.60	1.17	19.8	6.48	0.0001***		
$18^{\circ}\text{C}$ -1ppt vs $18^{\circ}\text{C}$ -6ppt	9.43	1.12	21.4	8.45	< 0.0001***		
$21 ^{\circ}\text{C}$ -0 ppt vs $21 ^{\circ}\text{C}$ -1 ppt	-2.19	1.12	19.8	-1.86	0.64		
21 °C -0ppt vs 21 °C -6ppt	1.74	1.12	21.4	1.56	0.81		
21 °C -1ppt vs 21 °C -6ppt	3.93	1.22	21.8	3.23	0.075		
$25 ^{\circ}\text{C}$ -0ppt vs $25 ^{\circ}\text{C}$ -1ppt	0.57	1.17	19.8	0.48	1		
$25 ^{\circ}\text{C}$ -0ppt vs $25 ^{\circ}\text{C}$ -6ppt	5 90	1 11	17.4	5 32	0.0013**		
$25 ^{\circ}\text{C}$ -1ppt vs $25 ^{\circ}\text{C}$ -6ppt	5.34	1.21	18.3	4.41	0.0077*		
$18^{\circ}\text{C}$ -0ppt vs $21^{\circ}\text{C}$ -0ppt	2.56	1 17	19.8	2.19	0.45		
$18^{\circ}C$ -0ppt vs $25^{\circ}C$ -0ppt	1 79	1.17	19.8	1.52	0.83		
$21 ^{\circ}\text{C}$ -0 ppt vs $25 ^{\circ}\text{C}$ -0 ppt	-0.78	1.07	19.0	-0.73	1		
$18^{\circ}\text{C}$ -1ppt vs 21 °C -1ppt	2.20	1.22	21.8	1 81	0.67		
$18^{\circ}C$ -1ppt vs $25^{\circ}C$ -1ppt	4 18	1.22	21.8	3 44	0.05*		
$21 ^{\circ}\text{C}$ -1ppt vs $25 ^{\circ}\text{C}$ -1ppt	1.97	1.27	20.3	1 56	0.82		
$18^{\circ}\text{C}$ -6ppt vs $21^{\circ}\text{C}$ -6ppt	-3 29	1.127	21.4	-2.95	0.13		
$18^{\circ}\text{C}$ -6ppt vs $25^{\circ}\text{C}$ -6ppt	0.086	1 11	17.4	0.077	1 00		
$21 ^{\circ}\text{C}$ -6ppt vs $25 ^{\circ}\text{C}$ -6ppt	3 38	1 15	19.5	2 93	0.14		
	5.50	Thermal Safety	Margin	2.75	0.14		
		Temperati	17.141 5111 17.0				
		mpcrui	·· ·				

18 °C vs 21 °C	-1.92	0.41	NA	-4.68	<1e-04***
18 °C vs 25 °C	-2.61	0.41	NA	-6.39	<1e-04***
21 °C vs 25 °C	-0.70	0.37	NA	-1.87	0.15
		Chloride	ę		
Oppt vs 1ppt	0.74	0.42	NA	1.76	0.18
Oppt vs 6ppt	0.94	0.41	NA	2.29	0.057
1ppt vs 6ppt	0.20	0.39	NA	0.50	0.87
		Interactio	ns		
18 °C -0ppt vs 18 °C -1ppt	-0.74	0.43	21.8	-1.74	0.72
18 °C -0ppt vs 18 °C -6ppt	0.70	0.38	19.0	1.86	0.65
18 °C -1ppt vs 18 °C -6ppt	-0.20	0.39	21.4	-0.50	0.99
21 °C -0ppt vs 21 °C -1ppt	-1.07	0.41	19.8	-2.59	0.25
21 °C -0ppt vs 21 °C -6ppt	-0.098	0.39	21.4	-0.25	1.00
21 °C -1ppt vs 21 °C -6ppt	0.97	0.43	21.8	2.27	0.40
25 °C -0ppt vs 25 °C -1ppt	-0.18	0.41	19.8	-0.45	1.00
25 °C -0ppt vs 25 °C -6ppt	-0.35	0.39	17.4	-0.90	0.99
25 °C -1ppt vs 25 °C -6ppt	-0.27	0.43	18.3	-0.40	1.00
18 °C -0ppt vs 21 °C -0ppt	1.92	0.41	19.8	4.64	0.0041**
18 °C -0ppt vs 25 °C -0ppt	2.61	0.41	19.8	6.34	0.0001***
21 °C -0ppt vs 25 °C -0ppt	0.70	0.38	19.0	1.86	0.65
18 °C -1ppt vs 21 °C -1ppt	1.59	0.43	21.8	3.72	0.027*
18 °C -1ppt vs 25 °C -1ppt	3.17	0.43	21.8	7.42	<0.0001***
21 °C -1ppt vs 25 °C -1ppt	1.58	0.45	20.3	3.55	0.041*
18°С -бррt vs 21°С -бррt	2.76	0.39	21.4	7.02	<0.0001***
18 °C -6ppt vs 25 °C -6ppt	3.20	0.39	17.4	8.20	<0.0001***
21 °C -6ppt vs 25 °C -6ppt	0.44	0.41	19.5	1.09	0.97

Asterisks denote significant p values (\*\*\* < 0.001; \*\* < 0.01; \* < 0.05)

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## GENERAL CONCLUSIONS

Predictive risk assessment of invasive species impacts is challenged by the influence of multiple overlapping stressors imposed by human activities. Multiple stressors can have additive or interactive effects that modify species performance and impact within a given ecosystem (Matthaei & Lange, 2016). Yet, very few studies have investigated these contexts for goldfish, one of the world's most widespread invaders (see Chapter 1). Goldfish are most often released into urban ponds (Copp et al., 2005), which could act as invader "boot camps" by imposing selective filters that shape the acclimation or adaptation potential of fish to other humandisturbed waterways. Invader performance could be enhanced by acclimation to a stressor in environmental conditions that overlap with goldfish physiological optima. Rural areas are increasingly homogenizing to the stressors present in urban counterparts, although perhaps at a slower rate (Philip et al., 2022). For instance, rising water temperatures, declines in dissolved oxygen, and increasing chloride pollution have been predicted or observed for Canadian surface waters including the Great Lakes-St. Lawrence River basin (Dugan & Arnott, 2023; Iacarella et al., 2015; St-Hilaire et al., 2016; Tellier et al., 2022). An important question for predictive risk assessment is how goldfish and other aquatic invasive species will perform under these rapidly changing conditions.

By measuring comparative functional response (Chapter 2), critical thermal maxima (Chapter 3) and associated metrics, I showed that realistic (environmentally relevant) levels of warming and chloride pollution significantly influence the feeding efficiency and thermal performance of cultivated (pet store) goldfish. The interaction of temperature and chloride affected the foraging behaviour of goldfish. I found support for the environmental matching hypothesis (Iacarella et al., 2015) and the metabolic theory of ecology (Iacarella et al., 2015; Volkoff & Rønnestad, 2020; Walker et al., 2020) in explaining these responses. Goldfish per capita effects are exacerbated by warming, and current levels of salinization can interact with temperature by dampening effects at low temperatures and amplifying effects at high temperatures. These findings highlight the need to account for multiple context dependencies in a framework to predict impact, as isolating one stressor can yield misleading results.

Similar effects of combined stressors were characterized for the thermal tolerance of goldfish. Depending on the metric investigated, temperature and chloride individually mediated

(i.e. CTmax, TSM) and interacted to mediate (i.e. Tag, Taw, Aaw) thermal capacity with acclimation. The incorporation of intermediate treatment levels (Chapter 3) allowed me to capture the buffering effects of realistically high chloride on thermal tolerance at sub-optimal temperatures. CTmax results agree with the prediction that both optimal temperature and chloride acclimation would enhance thermal tolerance; however, the effects of optimal and suboptimal temperatures were more pronounced than optimal chloride levels. I show that reported physiological optima predicted to yield high impact are less accurate when stressors interact. Overall, both sets of experiments reveal the direction and relative magnitude of the potential trophic impact of introduced goldfish if rural waterbodies homogenize to the levels of stressors currently altering urban ecosystems.

## Future directions

There are limitations to extrapolating the interactions detected in lab-based experiments to real ecosystems. Obviously, the functional response experiments fail to capture biotic complexities such as competition between species, non-consumptive effects of higher-order predators, and food resource switching. Nonetheless, functional response metrics have been shown to be robust in providing predictive information for field impacts (e.g., Cuthbert et al., 2019). For greater extrapolation power, the next step would be to compare results from cultivated pet-store goldfish to wild goldfish from established urban and rural populations, which could help identify selective traits in invasive populations. For management purposes and early prevention, it would also be useful to gain insight into competition outcomes by comparing invaders against native populations and native prey items (Dickey et al., 2022; Guo et al., 2017). Comparisons of functional responses suggest that goldfish have the potential to outcompete native species (Dickey et al., 2022), but this potential varies with the focal prey item (Guo et al., 2017). Finally, to achieve accurate risk assessments in rapidly changing environments over a range of aquatic ecosystems, it would be useful to examine the influence of a variety of multiple stressors (such as those summarized in Chapter 1)—in particular, oxygen stress alongside warming and salinization.

Overall, my thesis provides insight into the potential for stressors to mediate some fundamental responses (resource consumption and thermal acclimation) that can shape invasion success and impact. Rapidly changing conditions in urban ponds may select, acclimate or adapt plastic species such as goldfish to conditions that increasingly become the norm for other, more 'natural' waterways.

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