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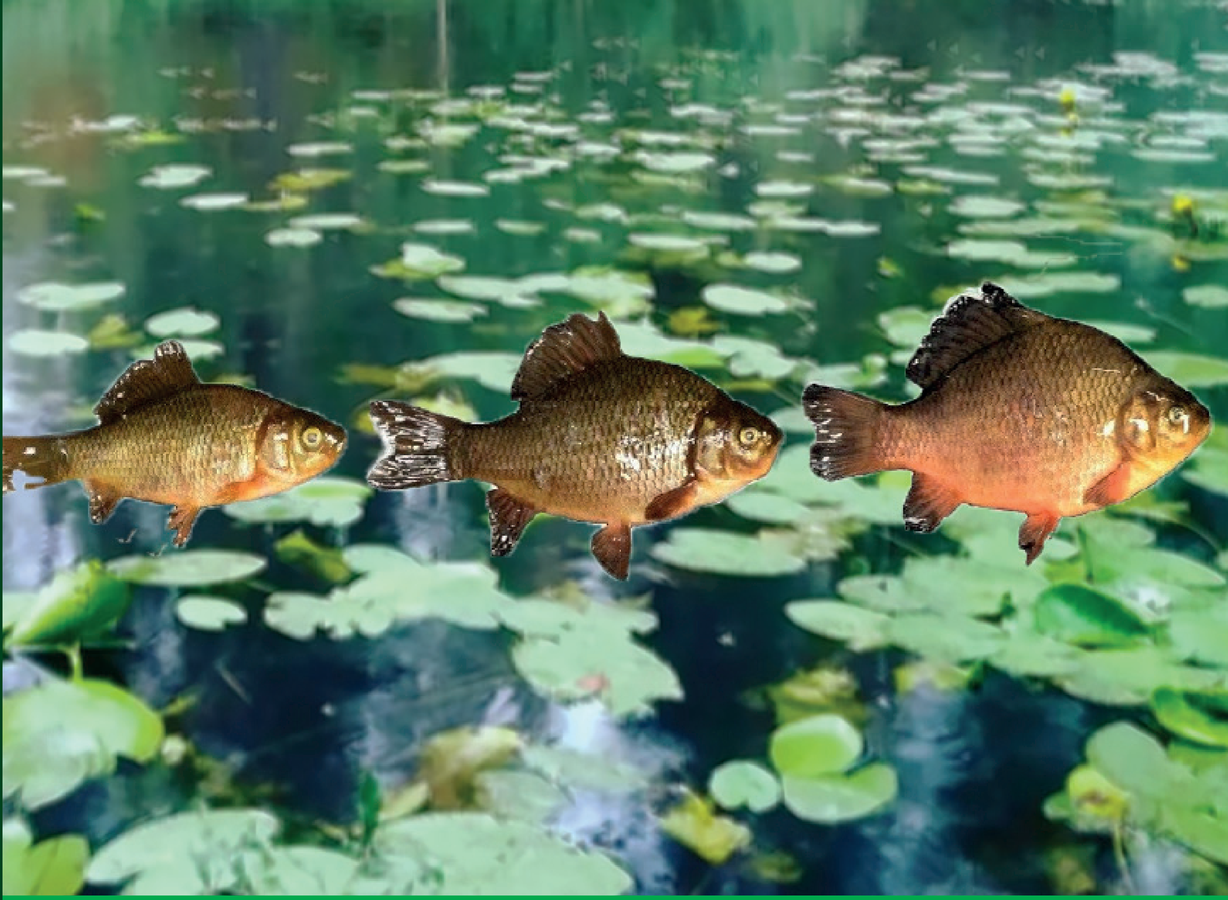

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

Ilaria de Meo

Ecological mechanisms driving the anti-predator defense response in Crucian carp *(Carassius carassius)*

Variation in morphology, resource use, and life-history strategies along a gradient of predation risk

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Author contact

Ilaria de Meo
Inland Norway University of Applied Sciences, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Campus Evenstad, 2480 Koppang, Norway
E-mail: ilaria.demeo@outlook.it

Primary supervisor

Antonio Bjørn Stefano Poleo
Inland Norway University of Applied Sciences, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Campus Evenstad, 2480 Koppang, Norway

Co-supervisor

Kjartan Østbye
Inland Norway University of Applied Sciences, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Campus Evenstad, 2480 Koppang, Norway

Abstract

Predation is one of the main structuring forces of freshwater communities, influencing population dynamics, phenotypic variation, resource use, and life-history traits within and among prey populations. In order to counteract predation risk, prey organisms may display several anti-predator morphological and behavioral adaptations. The crucian carp (*Carassius carassius*) represents a classic example of predator-induced morphological defense, as it develops a deep body to decrease vulnerability to predation. Very few studies, however, have explored the ecological drivers underlying morphological variation observed among crucian carp populations in the wild. This PhD thesis aimed at revealing the effects of predation risk on morphology, resource use, and life-history traits of crucian carp along a natural gradient of predation risk. The study was performed in fifteen small lakes from southern Norway, which presented no predators or increasingly efficient gape-limited predators: brown trout, perch, or pike. The results show that crucian carp is provided with a fine-tuned morphological defense response against gape-limited predators. Progressively efficient predators determined an increase in crucian carp relative body depth and size, a decrease in population density, rapid growth at young age, larger lifespans, and higher reproductive effort. Predation pressure likely reduced fish abundance, relaxing intraspecific resource competition and favoring individual growth of survivors. High-predation lakes also corresponded to productive systems with high food availability and complex vegetated littoral habitats. Reduced intraspecific competition, larger food availability, and increased habitat complexity associated with predation risk favored energy allocation to both growth and reproduction. Plastic feeding habits and a shift to more energetically rewarding prey with increasing body size may also have supported this energy allocation. Thus, the expression of the defense response in crucian carp was likely a result of the synergistic effects of predation risk and favorable environmental conditions.

Keywords: Crucian carp, body depth, predator-induced morphological defenses, lake productivity, generalist fish, life-history traits**.**

Sammendrag

Predasjon er en av de viktigste kreftene som strukturerer økosystemene i ferskvann, gjennom sin effekt på populasjonsdynamikk, fenotypisk variasjon, ressursbruk, og livshistorietrekk mellom og innen populasjonene av byttedyr. For å redusere risikoen for å bli spist kan byttedyrene utvise mange ulike antipredator-strategier, som for eksempel morfologiske og atferdsmessige tilpasninger. Utviklingen av en høy kroppsform hos karuss (*Carassius carassius*) er et klassisk eksempel på en predatorindusert morfologisk tilpasning (forsvar mot predasjon) for å unngå å bli spist av rovfisk. Det er imidlertid få studier som har undersøkt hvilke økologiske drivere som ligger bak den morfologiske variasjonen vi finner mellom ulike karusspopulasjoner i naturen. Denne PhD-avhandlingen har hatt som mål å finne ut hvordan predasjonsrisiko langs en naturlig gradient påvirker karussens morfologi, ressursbruk og livshistorietrekk. Studien ble gjennomført i femten små karussvann i Østlandsområdet, som varierte fra ikke å ha predatorer til stede til å ha predatorer med en økende grad av effektivitet (basert på størrelsen til fiskens gap): brun ørret (*Salmo trutta*), abbor (*Perca fluviatilis*) og gjedde (*Esox lucius*). Resultatene viser at karuss har en fininnstilt morfologisk forsvarsrespons mot rovfisk, som henger sammen med størrelsen på gapet til predatoren. Med økende effektivitet (gap) hos predatoren økte karussen i størrelse og relativ kroppshøyde, populasjonstettheten sank, veksten ble raskere i ung alder, livslengden økte og den reproduktive innsatsen økte. Det ser ut som om predasjonspress reduserer forekomsten av karuss, letter den intraspesifikke konkurransen om ressurser og favoriserer den individuelle veksten hos de som overlever. De vannene som hadde den høyeste graden av predasjon var også de mest produktive med høy tilgang på føde og med komplekse habitater i form av omfattende strandvegetasjon. Predasjonsrisiko ga redusert intraspesifikk konkurranse, mer tilgjengelig føde og økende habitatkompleksitet. Dette førte til mer energi til både vekst og reproduksjon. Varierende fødehabitat og et skifte til mer energirik føde som følge av økende kroppsstørrelse kan også ha bidratt til denne omfordelingen av energi. Det er altså sannsynlig at karussens forsvarsrespons er et resultat av synergi mellom effekten av predasjonsrisiko og fordelaktige miljøbetingelser.

Nøkkelord: Karuss, kroppshøyde, morfologisk forsvarsrespons mot rovfisk, produktivitet, generalist fisk, livshistorietrekk.

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de Meo I., Østbye K., Kahilainen K. K., Hayden B., Fagertun C. H. H., Poléo A. B. S. (2021). Predator community and resource use jointly modulate the inducible defense response in body height of crucian carp. *Ecology and Evolution 11*(5), 2072-2085. https://doi.org/10.1002/ece3.7176

Paper II

de Meo I., Østbye K., Kahilainen K. K., Hayden B., Magnus M., Poléo A. B. S. The resource use of Crucian carp along a productivity gradient reveals dynamic adaptations contingent upon body size, predation risk, and competition. *Manuscript.*

Paper III

de Meo I., Østbye K., Kahilainen K. K., Poléo A. B. S. Predation risk is the major driver of life-history changes of crucian carp in small boreal lakes. *Manuscript.*

1. Introduction

Predation has a central place in evolutionary ecology and plays a key role in the functioning and structuring of freshwater ecosystems, influencing not only population dynamics but also phenotypic variation, resource use, and life-history traits within and among populations (Sharma & Borgstrøm, 2008; Nosil & Crespi, 2006; Öhlund et al., 2020). At the individual level, predation affects almost every aspect of the prey organism's life, such as foraging, mating, and habitat selection. Predators can directly kill their prey, causing an abrupt decline in prey fitness, as they will never reproduce again. Alternatively, they can frighten their prey, which results in morphological, behavioral, and life-history adaptations to counteract predation risk (Tollrian & Harvell, 1999; Roff, 2002). These indirect, non-consumptive effects of predation involve energetic trade-offs which can affect prey fitness dramatically (Lima & Dill, 1990). Thus, prey populations need to optimize these trade-offs in presence of predators where both a plastic and more genetically hardwired strategy may evolve in the prey (Edgell et al., 2009). Predators are also subject to strong selective pressures for increasing their hunting efficiency, as they must capture enough food to survive and reproduce. So, why are prey not driven to extinction by predators with increasingly effective adaptations? According to the "life-dinner principle" (Dawkins & Krebs, 1979), in predator-prey interactions, prey are struggling for life while predators are trying to get their dinner. That means that a single mistake can have lethal consequences for the individual fitness of prey, while an unsuccessful hunting event does not prevent predators from surviving and reproducing. Thus, this asymmetry in selection pressure strength between predator and prey would keep the prey ahead in this 'evolutionary arms race'. This co-evolution has determined an extraordinary variety of predator and prey adaptations, such as morphological structures, crypsis, sensory systems, and avoidance and detection behaviors (Kishida & Nishimura, 2005; Teplitsky et al., 2005; Ferrari et al., 2010).

1.1. Adaptations for foraging

Predators have several structural, functional, and behavioral adaptations to increase their chance to detect, capture, kill and ingest prey. The efficiency of visual, chemical, and morphological adaptations for detecting prey in the aquatic environment is also strongly dependent on changes in abiotic conditions (Fraser & Metcalfe, 1997; Jönsson et al., 2013).

Water color and turbidity levels, for instance, can reduce foraging ability and determine shifts in prey selection, with potential effects on fish growth and interspecific interactions (Engström-Öst & Mattila, 2008; Ranåker et al., 2014; Scharnweber et al., 2016). Predators can exhibit a variety of foraging behaviors, which are also dependent on habitat characteristics. For example, piscivorous northern pike (*Esox lucius*) presents a sit-and-wait predation strategy, waiting for the moment to strike on prey from the vegetation (Skov & Nilsson, 2018), and tends to prey upon non-vigilant individuals (Heynen et al., 2017). On the opposite, perch (*Perca fluviatilis*) search actively for prey and select mainly mobile individuals (Heynen et al., 2017), but can switch to a sit-and-wait foraging mode with increasing habitat complexity (Eklöv & Diehl, 1994).

The ability of predator species to forage on prey from different habitats also depends on their morphological and anatomical features. For instance, a body that is laterally compressed provides fish with greater maneuverability to forage invertebrates associated with the substrate or vegetation. In contrast, a streamlined body shape enables fish to swim fast and efficiently catch their prey in the pelagic habitat (Webb, 1984). Variations in morphology associated with foraging efficiency in different habitats have also been observed between and within populations of the same species, such as in perch (Svanbäck & Eklöv, 2003), stickleback (*Gasterosteus* spp.; Schluter, 1993), and bluegill sunfish (*Lepomis macrochirus*; Ehlinger, 1990), where the littoral morph presented a deeper body compared to the pelagic one. These differences in foraging morphology can be genetically determined, environmentally induced or both, and, allowing populations to occupy different ecological niches, may promote divergent natural selection (Schluter, 1993; Smith & Skúlason, 1996; Skúlason et al., 2019).

1.2. Prey selection

Many predators feed on prey according to their availability, without showing a specific preference. Others are specialized to feed on specific prey items and present a narrow diet breadth. A more generalized or specialized feeding behavior will depend on the optimal forage strategy of the consumer. Optimal foraging theory states that consumers rank alternative resources by their energetic and nutritional value, taking into account the costs in energy and time spent in foraging (Werner & Hall, 1974). For example, piscivorous fish such as pike are gape-size limited and prefer to select small and shallow-bodied prey, since handling time increases with prey body depth (Nilsson & Brönmark, 2000). The pumpkinseed sunfish (*Lepomis gibbosus*) is a specialized molluscivore, but at high fish densities snail abundance is kept so low that they are forced to feed on soft-bodied littoral invertebrates, with effects on growth and morphology (Osenberg et al., 1992). High intraspecific competition can lead conspecifics to adopt different foraging behaviors and use distinct subsets of population diet breadth as preferred prey items become less available, resulting in an increase in population niche size (Svanbäck & Bolnick, 2007). At the same time, the ability of a consumer to forage on alternative resources is strongly dependent on interspecific competition (Bolnick et al., 2010). Resource partitioning generally constraints niche width by limiting the range of available resources to consumers, with cascading effects on trophic interactions (Quevedo et al., 2009; Eloranta et al., 2013). Thus, the trophic niche of an individual will depend on the abundance and diversity of available resources, morphological and behavioral adaptations to capture, handle and digest the prey, but also on the counteracting effects of intra- and interspecific competition.

Moreover, prey selection may vary between sexes. Variation in resource use between males and females can be the result of several mechanisms such as different activity levels or behaviors in response to predation (Estlander & Nurminen, 2014), energetic costs associated with growth and reproduction (Henderson et al., 2003), or sex-specific selective pressures (Höök et al., 2021).

Changes in prey preference can also occur over the lifespan of an individual. Perch, for example, can undergo two main ontogenetic dietary shifts: at the juvenile stage, they feed mainly on zooplankton; then, with increasing body size, there is a gradual shift to a benthivorous diet and finally to piscivory (Hjelm et al., 2000). Brown trout (*Salmo trutta*) is a generalist fish, but can also shift to piscivory at large sizes (Jensen et al., 2012). Besides body-size-related constraints limiting fish to feed on certain prey items during their lifetime, resource competition and predation risk are considered important factors affecting fish trophic ontogeny as they influence habitat use and thus prey availability to consumers (Sánchez-Hernández et al., 2019).

1.3. Non-consumptive effects

Predators can exert both consumptive and non-consumptive effects on prey. Direct consumptive effects have fatal consequences on the fitness of individual prey. By substantially reducing prey abundance, predators can also regulate resource availability and affect the size structure of prey populations (Persson et al., 1996; Van Buskirk & Yurewicz, 1998; Heibo & Magnhagen, 2005). The sole presence of predators can determine non-consumptive effects on prey that include the development of morphological, functional, and behavioral responses to counteract predation risk (Lima & Dill, 1990). Predation risk can induce the development of morphological defenses such as plates, shells, and spines that discourage attacks by predators (Tollrian & Harvell, 1999; Zimmerman, 2007) or an increase in body depth (Brönmark & Pettersson, 1994).

Antipredator behaviors include area avoidance, reduction in activity, and increased use of spatial and temporal refuges (Metcalfe et al., 1999; Ringelberg, 2009; Magnhagen et al., 2012). The choice of a certain behavior in prey is highly dependent on the predation strategy present (Peckarsky & McIntosh, 1998; Wood & Moore, 2020), habitat characteristics (Brydges et al., 2008; Ranåker et al., 2014), and food availability (Anholt & Werner, 1995). Morphological and behavioral responses to predation can also have strong effects on community dynamics. Large fish that have reached a size-refuge from predators may have a potential competitive advantage over smaller conspecifics or be able to shift to a different habitat (Byström et al., 2004; Woodward & Hildrew, 2002). Habitat shifts can lead to resource partitioning, since different prey species may be forced to use the same area, or selection of an unfavorable refuge as the preferred one is already occupied (Mittelbach, 1988; Henseler et al., 2020). These non-consumptive effects aimed at reducing immediate individual risk, involve time and resource allocation trade-offs and, thus, can indirectly affect prey fitness (Steiner & Pfeiffer, 2007). A high foraging activity, for instance, rewards an individual with more food, but can lead to greater exposure to predators. By contrast, a decrease in foraging activity lowers predation risk but can come with a cost for growth and reproduction, also prolonging the time spent within a predation window. The balance of these costs and benefits generates a trade-off between foraging gain and predation risk that is common in many prey organisms (Pettersson & Brönmark, 1993; McPeek et al., 2001; Benard, 2004).

1.4. Life-history strategies under predation risk

In fish, there is a clear trade-off between allocation to somatic growth and reproduction: fecundity increases with body size, but postponing maturity may lead to potential mortality before first reproduction (Roff, 2002). In general, life-history theory predicts that fast growth and high mortality should lead to early age and size at maturity (Stearns, 1992). Moreover, individuals with high reproductive allocation early in life typically have shorter life spans, because no energy for growth or surviving is left after reproduction (Bell, 1980; Charnov, 1993).

Predation has a central role in determining prey life-history strategies (Belk & Hales, 1993) and is expected to influence prey life-history patterns depending on prey size preference of predators (Abrams & Rowe, 1996). Mortality on small or young individuals generally favors late maturity and lower reproductive effort, while the opposite is expected with increased mortality on large or old individuals (Reznick et al., 1990). In the case of gape-limited predators, size-selective mortality on small individuals may favor preys growing rapidly into a size-refuge (Taborsky et al., 2003). This rapid somatic growth may be achieved by postponing reproduction until a safe size is reached (Belk, 1995; Arendt & Wilson, 1999). For instance, Trinidadian guppies (*Poecilia reticulata*) from populations with different predators showed remarkably different life-history patterns: in presence of a predator feeding on small size classes, guppies matured at a later age and larger size and had low reproductive investment compared to guppies occurring with a large predator feeding mostly on adult individuals (Reznick & Endler, 1982).

1.5. Predator-induced morphological defenses

The majority of defense traits are constitutive: they are always expressed regardless of predation risk. Many organisms, however, are able to adjust their phenotype to the prevailing risk of predation only when required (Tollrian & Harvell, 1999). Chemical cues released by predators (kairomones) or from damaged prey tissue can be detected by prey, inducing the development of defensive features that reduce vulnerability to predation (Wisenden, 2003; Ferrari et al., 2010). These predator-induced morphological defenses represent an adaptive phenotypic change in response to predation risk (Tollrian & Harvell, 1999). Pumpkinseed sunfish, for example, increase body depth and dorsal spine length in response to predation cues from walleye (*Sander vitreus*; Januszkiewicz & Robinson, 2007).

Prey organisms must often be able to face multiple predator species with different hunting modes (Sih et al., 1998). The freshwater crustacean genus *Daphnia*, for instance, can express a variety of defenses including the development of helmets, neckteeth, crests, and spines in response to different predators (Weiss et al., 2019; Figure 1.1). *Rana pirica* tadpoles can acquire a bulgy phenotype as a defense against the larval salamanders, while they express a "high-tail" phenotype in response to predation risk from dragonfly larvae, which bite their prey instead of swallowing it whole (Kishida & Nishimura, 2005; Figure 1.2). Although morphological defenses are widespread among freshwater organisms, few studies have explored how this morphological plasticity affects prey-population dynamics and their ecological role (Verschoor et al. 2004; Van der Stap et al., 2007; Kishida et al., 2009). At the individual level, for specific development of the appropriate defense, prey must be able to distinguish among predators through a specific sensory system and activate a fine-tuned "machinery" for the expression of the defense. Conversely, if the defense expressed is not effective against the predator, it may represent a disadvantage and reduce organism fitness.

The development of morphological defenses is considered a direct effect of predator presence (Tollrian & Harvell, 1999). However, the exact mechanism triggering the morphological change is not well understood. Some studies suggest that the expression of the defense is activated by a physiological stress response to predation risk (Middlemis Maher et al., 2013; Vinterstare et al., 2020). Other studies propose that inducible defenses are a by-product of prey behavior, since prey may reduce their activity levels with predation threat (Bourdeau & Johansson, 2012). This reduction in activity may lower prey metabolism with a reallocation of the energy saved to growth or development of morphological structures.

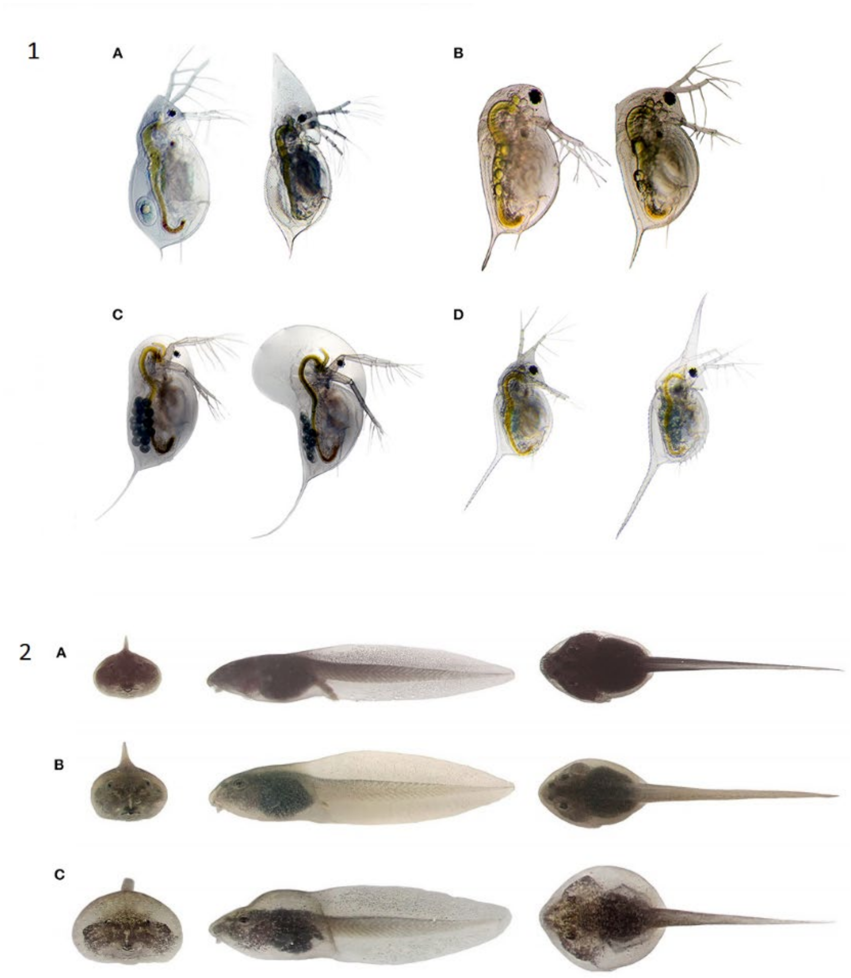

Figure 1. *Expression of predator-specific morphological defenses is highly flexible for the genus Daphnia (1). Examples show helmet expression in D. cucullata (1A), neckteeth expression in D. pulex (1B), crest expression in D. longicephala (1C), and head- and tail-spine formation in D. lumholtzi (1D). Undefended morphotypes are displayed on the left side, and the defended morphotype on the right side (Weiss et al., 2019). Rana pirica tadpoles (2) express basic morph in the absence of predators (2A), high-tail morph induced by the dragonfly (2B), and bulgy morph induced by the salamander (2C)(Kishida & Nishimura, 2005).*

1.6. The cost-benefit evolutionary framework of inducible defenses

In order to reduce the negative effects of predation on fitness, prey organisms display developmental, morphological, or behavioral antipredator defenses, which can be constitutive or inducible. When environmental conditions are stable, natural selection should favor populations to become locally adapted – that is, constitutive responses are favored when predation pressure does not vary in time (Williams, 2018). In contrast, inducible responses may improve prey fitness in environments with variable predation risk, when prey are able to reliably detect predator presence (Pigliucci, 2001). When the selection regime is stable over evolutionary time, inducible responses may be canalized and become genetically hardwired - i.e. always expressed regardless of predation risk (Edgell et al., 2009). The expression of the inducible response can also be more pronounced according to historical coexistence with a predator (Kishida et al., 2007). Moreover, transgenerational plasticity - parental exposure to predator cues directly affecting the phenotype of the offspring - may have an important role in the expression of inducible responses (Storm & Lima, 2010; Beaty et al., 2016).

In general, the induction of a plastic defense over a permanent one indicates that there are some fitness costs and benefits associated with the expression of the trait (Clark & Harvell, 1992; Snell-Rood et al., 2010). Inducible defenses can be particularly profitable as the intensity of the anti-predator response can be finely adjusted to the prevailing predation risk (David et al., 2014). At the same time, an inducible response might have potential energetic costs associated with the production and maintenance of the defense and the acquisition of information from the environment (DeWitt et al., 1998). These costs associated with the expression of the inducible defense are also dependent on environmental conditions. At low resource availability or high density of competitors, the investment in the defense is expected to be low, as energy is needed for maintenance or for actively foraging to avoid starvation (Werner & Anholt, 1993; Teplitsky & Laurila, 2007). Energy allocation to defensive traits may also have strong effects on fitness traits associated with growth and reproduction (Van Buskirk & Yurewicz, 1998; Brönmark et al., 2012). Numerous factors, not possible to be included in controlled laboratory studies, may influence the expression of plastic defense responses in the wild. Thus, measuring the fitness costs of predator-inducible defenses under more realistic ecological conditions is crucial for understanding how selection favors phenotypic plasticity.

1.7. Study species: the Crucian carp

Crucian carp (*Carassius carassius*) is a widespread cyprinid fish in Europe and Asia that inhabits small lakes, ponds, and slow-moving areas of rivers. It is a generalist forager with a wide diet spectrum, including zooplankton, benthic macroinvertebrates, macrophytes, and detrital material (Penttinen & Holopainen, 1992; Gao et al., 2017). This fish is well-known for its peculiar physiological adaptations. In autumn, crucian carp builds up a glycogen storage which uses for anaerobic metabolism under ice cover during winter (Blažka, 1958; Piironen & Holopainen, 1986). Since most other fish species are sensitive to oxygen depletion, a lake may change from a multispecies assemblage to a monospecific assemblage of crucian carp under severe winter conditions.

In lakes without predators, crucian carp are shallow-bodied, small-sized, and can reach very high densities, where resource limitation leads to strong intraspecific competition (Pettersson & Brönmark, 1997). By contrast, in lakes with piscivores, they are deep-bodied and typically occur in low density and large sizes (Brönmark et al., 1995; Poléo et al., 1995). This strong dichotomy in morphology, biology, and population structure, caused taxonomical confusion, as the two morphs were described as two different species, *Cyprinus carassius* and *Cyprinus gibelio* (Bloch, 1782). Then, in 1838, Ekström performed an experiment where deep-bodied crucian carp were moved to a small pond, and the offspring developed a population of small-bodied fish, suggesting that the two morphs were actually the same species. Later experiments showed that crucian carp develop a deep body when exposed to cues from piscivores feeding on conspecifics (Brönmark & Pettersson, 1994). This development was suggested to be an adaptive morphological defense against gape-limited predators (Nilsson & Brönmark, 2000). However, enhanced food availability and low population densities caused an increase in relative body depth comparable to predation risk (Tonn et al., 1994). Moreover, manipulative experiments showed that crucian carp feeding on benthic prey rather than zooplankton developed a deep body, similarly to the fish exposed to cues from predators (Andersson et al., 2006). In another experiment, crucian carp was exposed to pike cues (presence or absence) and different water currents (standing or running), and it was found that standing water conditions and predation cues independently induced a similar development in body depth (Johansson & Andersson, 2009). Whether these changes in growth and morphology are directly or indirectly mediated by predation, a deep body seems to be associated with a reduction in activity levels and better growth conditions (Holopainen et al., 1997a; Vøllestad et al., 2004). However, very few studies have explored the effects of predation risk on morphology, feeding strategies, and life-history traits of crucian carp in the wild.

2. Objectives

This PhD thesis aimed at revealing the main ecological drivers underlying morphological variation observed among natural populations of crucian carp. I considered fifteen small lakes from southern Norway along a gradient of predation risk represented by increasingly efficient gape-limited predators: no predator fish or brown trout, perch, or pike. I expected that increasing predation risk would induce specific morphological defenses – that is, progressively deeper body shape. I questioned if this change in body shape was caused by shifts in fish resource use or life-history strategies, driven by a direct or indirect response to predation risk. Moreover, I considered the synergistic effect of predation risk and other environmental factors such as lake productivity and resource competition, which vary in close association with the different predator communities.

This thesis addressed the following research objectives.

Variation in body shape (**Paper I).** We examined crucian carp from lakes that differ in predator fish communities (species composition, density, and gape-size), and we asked whether the prevailing risk of predation was associated with fish body shape. We also tested if variation in body shape depended on predator-induced shifts in habitat and resource use or other environmental factors such as productivity.

Variation in resource use (Paper II). We explored differences in the trophic ecology of crucian carp at the individual and population levels, considering the effects of intra- and interspecific competition, predation, and body size in lakes with different productivity and fish species composition.

Variation in life-history traits (Paper III). We examined variation in crucian carp life-history traits along a gradient of predation risk. We expected fish to attain a higher growth rate, larger adult size, later age and size at maturity, and lower reproductive effort with increasing predation risk.

3. Material and Methods

3.1. Study area and lake characteristics

The selected fifteen study lakes and ponds were located in southeastern Norway and were sampled between June and August in 2018 and 2019 (Figure 2, Table 1). Lakes were small (0.25–11 ha), relatively shallow (max depth 1.5–11.3 m), and presented a high proportion of littoral zone, characterized by high densities of macrophytes (mainly *Potamogeton* spp. and *Nuphar* spp.). Lakes had variable productivity, probably reflecting different land use of the surrounding area, including forest of birch or pine, forest with proximity to an urban area, urban areas, or farmland. Measured abiotic parameters included lake altitude, surface area, maximum depth, littoral area, specific conductivity, Secchi depth, total nitrogen, phosphorus, and organic carbon. Lakes presented distinct communities of piscivorous fish. Six lakes were

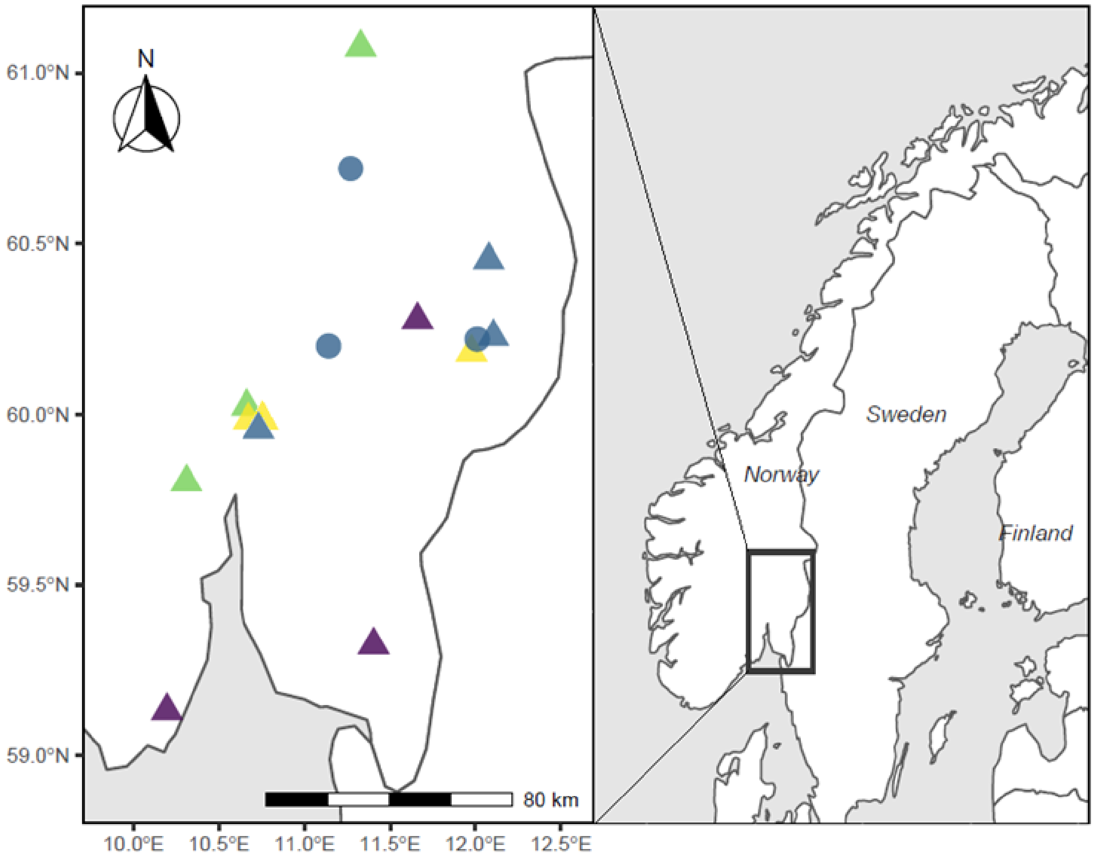

Figure 2. *Location of the fifteen study lakes in southeastern Norway. Triangles indicate lakes used in paper I and II. Circles indicate additional lakes used in paper III. Blue: no predator lakes; Green: brown trout lakes; Yellow: perch lakes; Purple: pike lakes.*

Table 1. *Environmental characteristics of the 15 study lakes. Abiotic variables include latitude (Lat), longitude (Long), lake area (Area), maximum depth (MaxD), total nitrogen (TotN), total phosphorus (TotP), total organic carbon (TOC), maximum predator gape size (GS) and crucian carp relative density (CPUEc).*

Lake	Predators	Lat (^{o}N)	Long (^{o}E)	Area (ha)	MaxD (m)	TotN (µg/L)	TotP (µg/L)	GS (cm)	CPUEc (n· net^{-1}h^{-1})
Nusttjennet	Pike, Perch	60.28	11.66	11.00	1.5	1090	164	4.7	0.45
Bugårdsdammen	Pike, Perch	59.13	10.19	5.04	2	980	54	6.1	0.10
Stomperudtjern	Pike, Perch	59.32	11.40	3.85	1.5	1660	146	5.6	0.42
Øvresetertjern	Perch, Trout	59.98	10.67	3.05	3.5	446	13	4.4	0.42
Posttjernet	Trout	61.08	11.33	1.72	11	312	8	3.8	0.19
Svartkulp	Perch, Trout	59.98	10.74	5.80	10	550	13	3.5	0.30
Bjørnmyrdammen	Perch	60.18	11.98	2.10	3.5	672	26	2.7	2.11
Småvanna	Trout	59.80	10.31	0.50	3.8	616	14	3.3	1.03
Karussputten	Trout	60.02	10.66	0.25	4.6	361	9	3.3	0.99
Skråtjernet	-	60.20	11.14	0.88	12.0	431	15	-	1.83
N.Sætertjern	-	60.22	12.01	0.57	2.5	-	-	-	7.93
Forkerudstjern	-	60.45	12.08	1.24	2.2	1985	82	-	10.63
Brynitjernet	-	60.72	11.27	4.29	1.5	572	22	-	4.08
Motjennet	-	60.23	12.11	0.94	11.3	688	23	-	7.80
Langmyrtjern	-	59.97	10.75	0.30	5	702	20	-	2.07

predator-free (Forkerudtjern, Mottjernet, Langmyrtjern, N.Sætertjern, Skråtjernet, Brynitjernet), while nine lakes had different gape-limited predators, which we grouped in three main predation categories in Paper I: brown trout, perch, and pike. Brown trout was the only predator in Karussputten, Småvanna, and Posttjernet. Perch was the main predator in Svartkulp, Bjørnmyradammen, and Øvreseterjern while brown trout was present with very low density or absent. Both perch and pike were present in Bugårdsdammen, Stomperudtjernet, and Nusttjennet, but we will refer to these lakes as “pike lakes” for simplicity. We included all fifteen lakes in paper III, while we excluded Lakes N.Sætertjern, Skråtjernet, and Brynitjernet from papers I and II due to cost and time considerations.

3.2. Fish and invertebrate sampling

We assessed relative fish density in each lake using Nordic multi-mesh gillnets. Some lakes had very limited pelagic and profundal habitats and were considered as entirely littoral. Moreover, we did not catch any fish in the profundal zone, probably because the deeper lakes were highly humic systems with hypoxic deep waters. Consequently, we calculated CPUE (*n* fish $\cdot \text{net}^{-1} \cdot \text{h}^{-1}$) only for littoral and pelagic habitats. We also used a variety of fishing methods (e.g., baited traps, gillnets with different mesh sizes, kick nets) to maximize the chance to catch small crucian carp. Immediately after capture, fish were euthanized by an overdose of the anesthetic tricaine methanesulfonate (MS-222), transported to the laboratory, and frozen for subsequent analysis. Permission to catch fish was granted by the Norwegian Environmental Agency (2018/4155).

We collected qualitative samples of zooplankton and benthic invertebrates in order to estimate basal resources for stable isotope analysis (SIA) of paper I and II. We sampled zooplankton hauling a plankton net through the water column in the pelagic zone of deep lakes or in the non-vegetated area of shallow lakes. Samples were later sieved and zooplankton were identified to class level. We collected benthic invertebrates from sediments and plants in the littoral habitat using kick nets and sorted them to the lowest feasible taxonomic level. For paper II, samples of sediment and of the most abundant macrophytes were also collected from the littoral zone of each lake. Sediment comprised primarily decaying plant material.

3.3. Laboratory work

In the laboratory, we measured individual fish total length, body depth, wet weight and determined sex and maturation stage of crucian carp by gonad inspection. We subsampled 30 crucian carp from each of the twelve lakes selected for paper I and II, for a total of 360 individuals. In general, crucian carp size structure was quite uniform within each lake and dominated by large individuals in lakes with predators. Thus, whenever possible, we included smaller individuals to avoid underrepresentation of this size class. These fish were used for morphometric (paper I), stable isotope (paper I and II) and diet (paper II) analysis. For stable isotope analysis, a piece of dorsal muscle tissue was dissected and frozen at -20°C for each fish. When possible, for diet determination, we included more individuals to increase sample size, since fish with empty guts were excluded from the analysis. We estimated the proportion occupied by different prey category observed in the anterior third of crucian carp gut under a stereo microscope using percentage scale. For life-history traits analysis (Paper III), we determined age by counting true annuli along the primary radius of scales ($n \sim 6$) using a Microfiche Reader (Eyecom 1000). In addition, for paper I and III, we measured total length and gape height of piscivorous fish, that is, brown trout, perch, and pike. We calculated the mean maximum gape size for each lake by selecting the piscivores with the highest mouth height, irrespective of the species ($n = 10$). We selected maximum gape height over mean gape height to avoid potential underestimation of maximum predation window since a large proportion of brown trout and perch in the study lakes was small-sized and probably did not undergo ontogenetic shifts to piscivory (Jensen et al., 2012; Hjelm et al., 2000).

3.4 Stable isotope and diet analysis (Paper I and II)

Fish muscle, invertebrate, plant, and sediment samples were freeze-dried, ground to a homogeneous fine powder, weighed, and encapsulated into tin cups. Stable carbon and nitrogen isotope ratios were analyzed by an elemental analyzer coupled to a continuous flow mass spectrometer. Stable isotope measurements were expressed as δ^{13}C and δ^{15}N in parts per thousand (‰) relative to the international standards Vienna Pee Dee Belemnite and atmospheric air for carbon and nitrogen, respectively.

In paper I, we standardized crucian carp δ^{13}C and δ^{15}N ratios by using littoral and pelagic invertebrates in each lake as baseline. Individual trophic position and littoral reliance (i.e. relative contribution of littoral prey items to crucian carp diet) were calculated using a two-source mixing model (Karlsson & Byström, 2005) with trophic fractionation values of 3.4‰ for δ^{15}N and 0.4‰ for δ^{13}C (Post, 2002). These values were then related to the body shape of crucian carp.

Since basal food isotope ratios varied greatly across lakes, in paper II, we estimated resource contribution to crucian carp diet through a different approach. We removed variation not associated with fish trophic changes rescaling raw isotope values of consumers into modified Z-scores using mean and standard deviation of their four major prey categories (pelagic zooplankton, pooled benthic macroinvertebrates, and pooled plant and sediment) at each location (Fry & Davis, 2015; Lejeune et al., 2021). Then, we performed generalized linear mixed effects models to test for the effects of body size, productivity, and relative density of crucian carp, competitor fish, and predators on individual $\Delta^{13}C$ and $\Delta^{15}N$ values calculated from the modified Z-scores. Selection of the best models was performed by backward elimination of fixed-effect terms based on the Akaike information criterion (AIC). To estimate the predominant resource use of crucian carp at the population level, we used Bayesian isotope mixing models (Parnell et al., 2010). In the model, we used trophic fractionation values of 3.4 ± 1.0‰ for $\delta^{15}N$ and 0.4 ± 1.3‰ for $\delta^{13}C$ (Post, 2002). Moreover, we calculated the isotopic niche width of crucian carp in each lake as the standard ellipse area corrected for small sample sizes (SEAc; Jackson et al., 2011).

For diet analysis of paper II, observed prey items from gut contents were grouped in three main categories: pelagic prey (mainly pelagic cladocerans and copepods), benthic prey (chironomid larvae, gastropods, benthic cladocerans and, other benthic invertebrates), and plant-detritus material. We quantified diet breadth at the population level using Levins' D index (Levins, 1968) standardized for resource richness. In addition, we assessed individual dietary specialization (IS) calculating the mean overlap between the diet of each individual to that of its population (Bolnick et al., 2002).

3.5 Association of body shape with environmental variables (Paper I)

We examined crucian carp body shape using landmark-based geometric morphometrics. We photographed fish laterally and digitized 17 landmarks and six semi-landmarks. Then, we used a Generalized Procrustes Analysis (GPA) to standardize the landmark configurations for position, orientation, and size. Principal component analysis (PCA) on Procrustes shape coordinates was used to identify the major patterns of shape variation and grouping of variance among individuals. A Procrustes ANOVA with permutation procedures was used to estimate allometric effects (i.e. shape variation in relation to size) among predation categories (no predators, trout, perch, and pike). Then, we used linear mixed-effects models (LME) to examine the degree of relationship between variation in body depth and specific biotic and abiotic characteristics associated with each lake. The scores of the first axis of principal component (PC1), which corresponded largely to the crucian carp relative body depth, were used as the response variable. Explanatory variables for predation risk included predation category, maximum predator gape size, and predator proportion. Littoral reliance and trophic position calculated from SIA were used as a measure of individual crucian carp resource use. Abiotic characteristics included lake area, maximum depth, and total phosphorus. Lakes were used as a random factor nested in the predation category term. Model selection was performed by stepwise selection based on the Akaike information criterion (AIC).

3.6 Determination of life-history traits (Paper III)

We estimated growth parameters using a Bayesian approach through the 'BayesGrowth' package (Smart & Grammer, 2021) in R statistical software. We fitted the von Bertalanffy growth function to the data from each lake. We used informative priors on the length-at-birth (L_0) and the asymptotic length (L_∞) parameters with a normal distribution. We ran four Markov-Chain Monte Carlo (MCMC) chains with 10000 iterations and a burn-in period of 5000 iterations via the 'rstan' R package (Stan Development Team, 2020). The length (L_M) and age (A_M) at which 50% of individuals became sexually mature was determined by fitting a logistic model to the proportion of mature versus immature using generalized linear models. Moreover, we calculated gonadosomatic index (GSI) for females as (gonad mass/somatic mass*100) (Bagenal & Tesch, 1978). Then, we correlated growth and reproductive parameters to predator maximum gape size. Growth parameters included the growth coefficient (k), asymptotic body length (L_∞), length at age 1 (L_1) and age 3 (L_3), and maximum life span (T_Max). Length at ages 1 and 3 was chosen as representative of initial growth effort to avoid predation. Maximum life span was calculated as the average maximum age observed in each population (n=10). Reproductive parameters comprised length (L_M) and age (A_M) at maturity, gonadosomatic index (GSI), and sex ratio. We also included the reproductive life span (RT) calculated as the difference between the observed maximum life span and estimated age at maturity.

4. Results and discussion

4.1. Variation in body shape: the role of predation risk and resource use (Paper I)

Variation in body shape

The body shape of crucian carp differed greatly among the study lakes. Shape variation along the first axis of the PCA of landmark configurations (45% of the total variance) was mainly associated with an expansion of fish dorsal and ventral regions, indicating an overall change in body depth. Fish grouped along this axis mainly according to the different predation categories (Figure 3). Moreover, results of Procrustes ANOVA showed that body shape was positively related to size, and varied in relation to size among different predation categories – e.g. small-sized fish from pike lakes had a deeper body than fish of the same size from lakes without predators.

Predation risk: predator species composition, gape-size, and density

Predator maximum gape size, individual trophic position and body length were the best variables explaining variation of crucian carp body shape (Figure 4). In general, in absence of predators, fish were smaller and had a slender body shape. Then, they gradually showed higher absolute and relative body depth when trout and perch were present, reaching the largest size and deepest bodies in pike lakes. Experimental studies showed that crucian carp increased in body depth when exposed to cues from single predator species such as perch or pike, and that the latter induced a more pronounced development (Brönmark & Pettersson, 1994). Moreover, crucian carp exhibited different behavioral and neural responses to skin extract from trout, perch, and pike (Lastein et al., 2012). Our findings suggest that crucian carp develop specific responses in presence of specific piscivorous fish species.

Predator maximum gape size was the best variable explaining variation of crucian carp body depth (PC1). Discerning between the effect of predator community and gape size might be

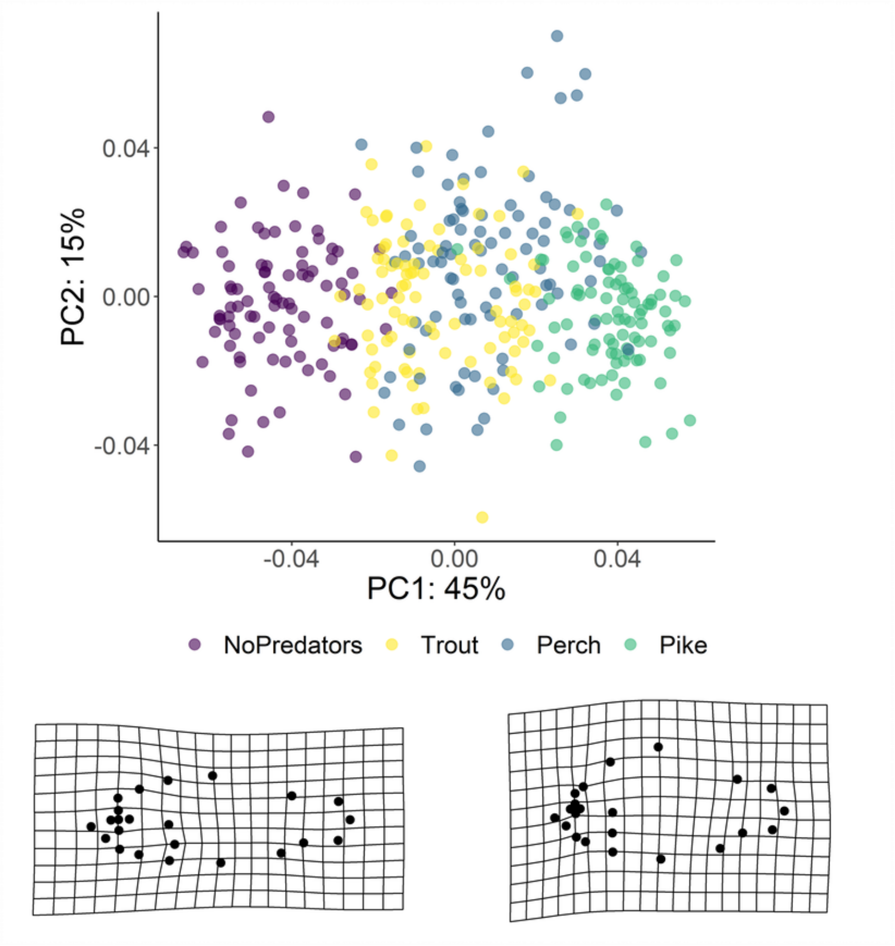

Figure 3. *Scatterplot of principal components analysis of body shape of crucian carp. Individuals are color-coded according to predation category. Deformation grids show the most extreme negative and positive shapes along the first (PC1) axis. Percentages indicate how much of the variation is explained by the first two axes.*

prevented by the fact that brown trout, perch, and pike lakes reflected a gradient in predation efficiency that was mainly defined by maximum mouth opening. Still, we could observe a clear effect of gape size even within predation categories. Perch in Lake Øvresetertjern and trout in Lake Posttjernet reached the largest gape size within their respective predation category. In these lakes, crucian carp had the highest body depth compared to the other lakes with the same predator species. Laboratory experiments showed that crucian carp was able to detect detailed information from waterborne cues. Individuals, for instance, showed different fright responses, as a decrease in swimming activity, when exposed to cues from predators of different sizes (Pettersson et al., 2000).

By contrast, predator density did not have a clear effect on body shape. In Lakes Stomperudtjernet and Nusttjennet, predators made up only a very small proportion of the total fish community (5%) and consisted of few large pike and perch. Nevertheless, in these lakes, crucian carp reached the largest size and deepest body. Pike, a highly efficient predator that shares the same vegetated habitat with crucian carp, represents a constant threat. Thus, in presence of pike, crucian carp might have developed an effective adaptive response to predation risk, independently from its density. These results support previous experimental studies suggesting that the development of a deep body represents a morphological defense against gape-limited piscivores (Nilsson & Brönmark, 2000).

Predator-induced shifts in resource use and availability

Contrary to our predictions, individual resource use varied greatly within each lake and did not have a strong direct effect on crucian carp body shape. Generally, fish seemed to rely mostly on littoral invertebrates associated with substrate or vegetation. By contrast, trophic position had a significant negative influence on crucian carp body depth. In pike lakes, crucian carp had slightly lower trophic position, probably because fish hiding in the vegetation may feed on macrophytes or other invertebrates such as snails or clams, which likely lowers their trophic position when compared to zooplankton feeding of other lakes. Moreover, crucian carp density was decreasing with increasing predation risk, which may have resulted in more available resources for the growth of surviving individuals (Persson et al., 1996; Craig et al., 2006). In contrast, dense populations in allopatric lakes have to compete for resources and fish body condition remains low. This also suggests a potentially higher growth rate with increasing predation risk. High productivity of pike lakes might also play an important role, since it regulates resource availability and ultimately population density and growth (Weber et al., 2010).

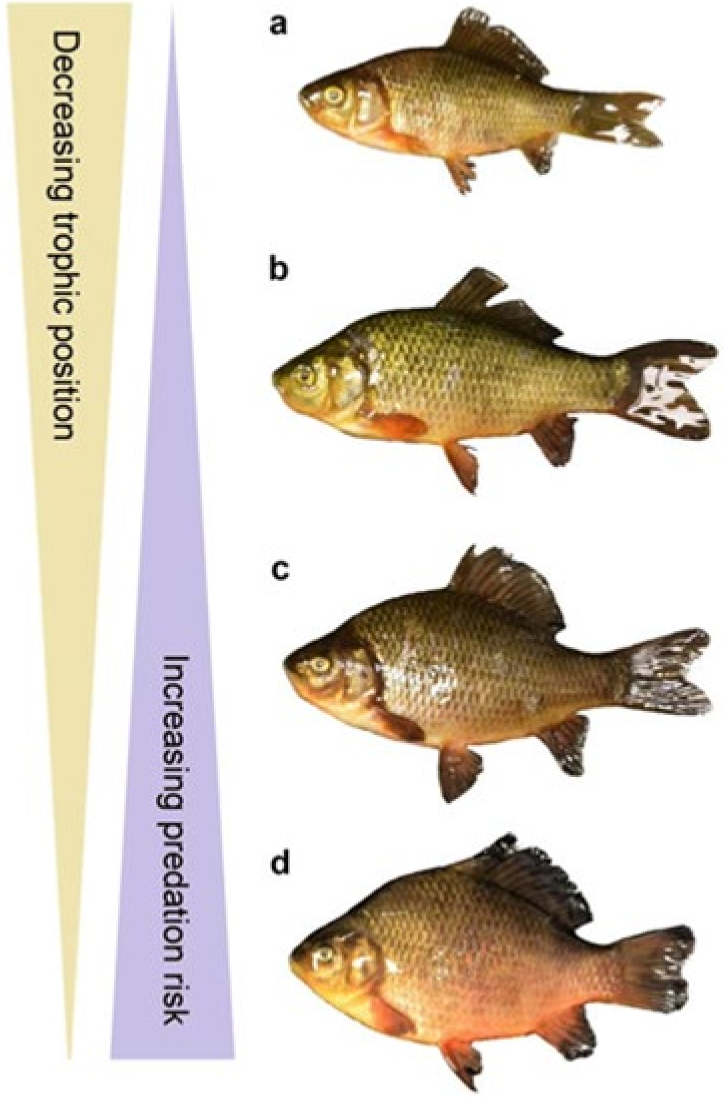

***Figure 4**. Crucian carp body depth increased with decreasing trophic position and increasing predation risk. In the figure crucian carp from lakes with different predation categories: no predators (a), brown trout (b), perch (c), and pike (d).*

4.2. Resource use along a productivity gradient (Paper II)

Shifts along a productivity gradient

Lake productivity represented one of the main drivers of crucian carp resource use and fish community structure. Stable isotope values of basal sources (littoral invertebrates, zooplankton, plants, and sediments) and crucian carp varied greatly among the different lakes, probably because of different land use of catchment area (Kendall et al., 2007; Botrel et al., 2014). Yet, even after accounting for this variation in the baselines, results from generalized linear mixed effects models showed a strong positive association of $\Delta^{13}C$ values with productivity. Analysis at the population level confirmed this pattern, as fish included higher proportions of detritus and plants in their diets in the most eutrophic lakes (Figure 5). Fish community structure was also shifting along the productivity gradient, as previously observed on broader spatial scales (Hayden et al., 2017). Crucian carp coexisted with brown trout, perch, or both brown trout and perch in mesotrophic lakes. Minnow (*Phoxinus phoxinus*) was also present in some of these lakes. The most productive lakes were sustaining multispecies communities including perch, pike, and high density of cyprinids such as roach (*Rutilus rutilus*), bream (*Abramis brama*), and rudd (*Scardinius erythrophthalmus*). In turn, high predation risk and large resource availability of productive lakes can determine an increase in crucian carp body size (Tonn et al., 1994). Thus, although explanatory variables were not directly correlated, productivity, community structure, and fish size were highly interconnected and likely had a synergistic effect on crucian carp resource use.

Size-related resource use

Body size was an important variable determining crucian carp resource use. In general, at the population level, fish seemed to include more littoral prey in their diet with increasing body size. At the individual level, we observed great variation in $\Delta^{13}C$ values of small fish, while $\Delta^{15}N$ values were lower relative to larger crucian carp. Consumption of few prey items such as filter-feeding zooplankton (mainly *Bosmina* sp.), detritus, or plant material and exclusion of large predatory benthic copepods or macroinvertebrates from the diet, could explain the low trophic position, high variation in $\Delta^{13}C$ values, and small niche size of crucian carp populations from allopatric lakes. By contrast, with increasing average body size, fish seemed to rely on

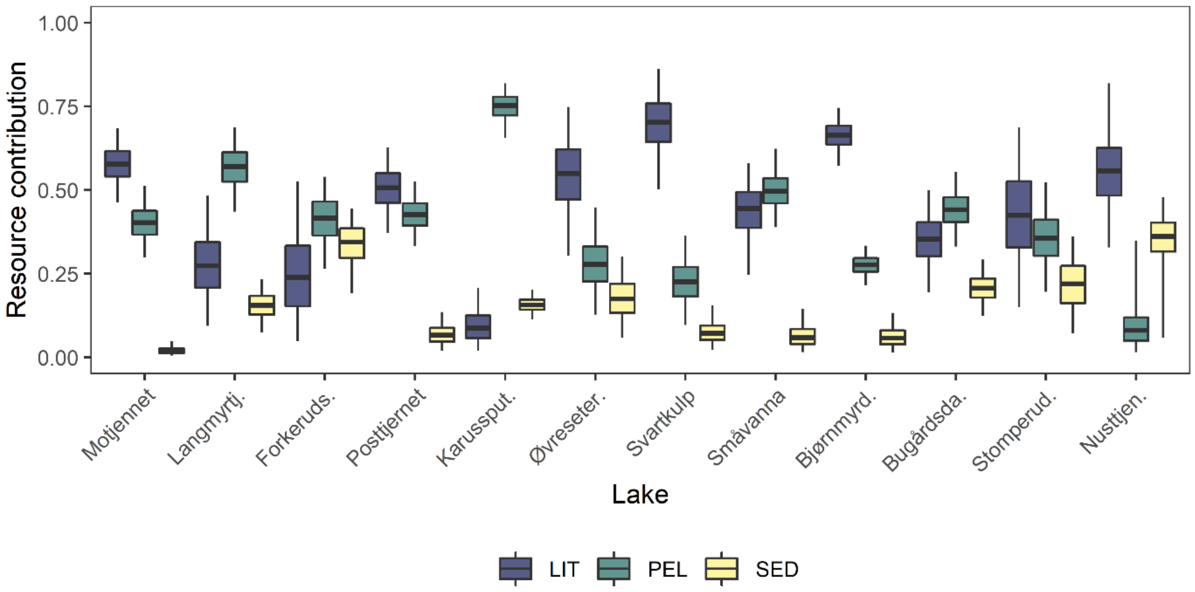

Figure 5*. Littoral (LIT), pelagic (PEL), and pooled plant and sediment (SED) resource use of crucian carp for each lake according to the Bayesian isotope mixing models. The box is drawn around the 25th and 75th quartiles, representing 50% credible intervals, while whiskers represent 95% credible intervals. Lakes are divided into communities without (1–-3) and with predators (4–-12) and arranged from left to right by increasing productivity within these categories.*

higher proportions of littoral prey. Diet analysis confirmed this pattern. Previous studies from allopatric populations reported that crucian carp preference for larger prey items increased with fish size (Penttinen & Holopainen, 1992). Experimental studies also showed that crucian carp present size-related shifts in feeding efficiency (Paszkowski et al., 1989). Thus, crucian carp may undergo changes in diet during ontogeny associated with size-specific differences in their ability to ingest prey.

Interspecific interactions

While crucian carp density was excluded from model selection, predator and competitor density were significant predictors of $\Delta^{13}C$ and $\Delta^{15}N$, respectively. In general, fish were more ^{13}C enriched in lakes with high predator density, probably because they were forced to feed on benthic prey when confined to the littoral area. By contrast, in absence of predators, crucian carp showed higher reliance on zooplankton, as they may use the pelagic area more freely (Pettersson & Brönmark, 1993; Diehl & Eklöv, 1995). Crucian carp, however, included a variable proportion of pelagic prey in their diet even in predator-lakes, probably because we mostly considered large individuals already outside of the predation window that were able to occasionally feed in the open water (Werner & Hall, 1988).

Interspecific competition also played an important role in determining crucian carp resource use. In the productive Lakes Nusttjernet and Stomperudtjernet, where high densities of different cyprinid species were present, crucian carp were ^{15}N depleted, had low individual specialization, and small isotopic niche area. Small bottom-dwelling cladocerans (mainly *Chydorus* sp.) accounted for more than 50% of their diet. Crucian carp may have been limited to feed on a restricted subset of the total prey spectrum, as a result of resource partitioning with other abundant cyprinid species such as roach and bream (Persson & Hansson, 1999; Nahon et al., 2020). In these productive lakes, predation risk and high density of competitor species may have decreased the degree of individual diet variation as a consequence of restricted habitat availability and limitation of alternative food sources (Eklöv & Svanbäck, 2006). However, fish managed to reach very large body sizes even in these lakes, likely because resources were abundant. In other lakes, competition with minnow might have limited crucian carp resource use as they cohabit in the littoral area (Eklöv et al., 1994). In lakes Bjørnmyrdammen, Øvresetetjern, and Bugårdsdammen, where no other cyprinid species was present, crucian carp were clearly ^{15}N enriched and included larger proportions of macroinvertebrates in their diet, indicating utilization of different resources compared to the other lakes.

4.3. Variation in life-history traits along a gradient of predation risk (Paper III)

Population structure

In absence of predators, fish populations consisted of abundant, small, and shallow-bodied individuals. By contrast, in presence of piscivores, catches were dominated by low densities of deep-bodied individuals, which were mostly well above predator gape size, especially in lakes where both perch and pike were present. Relative body depth of crucan carp was positively correlated with predator gape size (Figure 6).

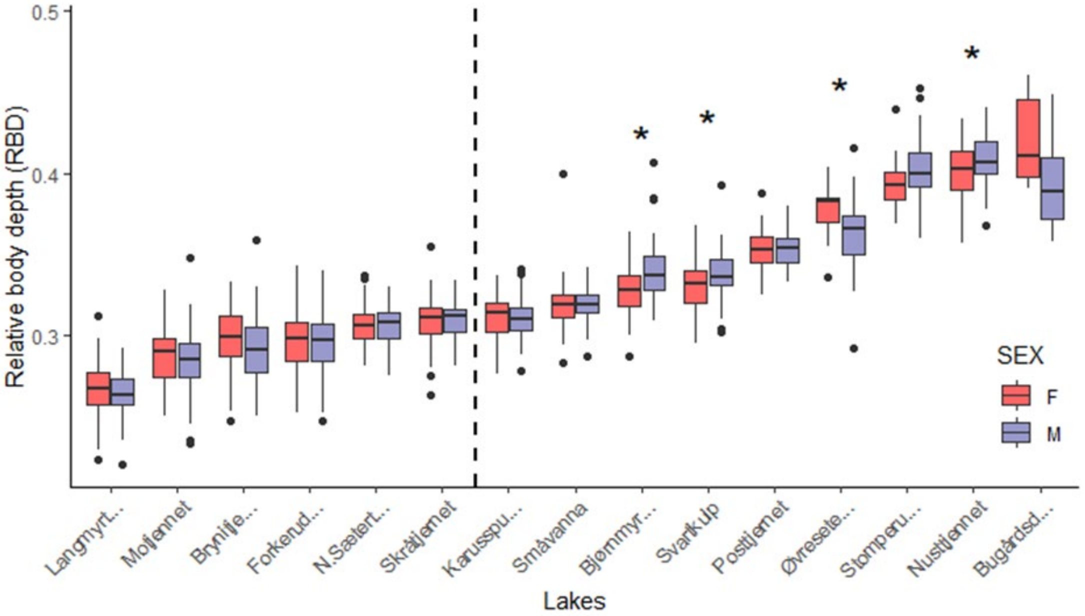

Figure 6. *Boxplots showing variation in relative body depth (RBD) between female (F) and male (M) crucian carp from the fifteen study lakes. Asterisks (*) denote significant differences (p < 0.05) between females and males. The dashed line separates lakes without predators (left side) from lakes with predators (right side).*

Growth traits

Mean parameter estimates from the von Bertalanffy growth function varied greatly among the various lakes (Figure 7). L_∞ ranged from 12.6 cm to 42.0 cm and was positively correlated with predator gape size. Young crucian carp also attained larger body length with increasing predator gape size, showing clearly a higher growth rate during their first years of life in presence of predators. These results show that fish can quickly achieve a size-refuge by increasing both in body depth and size, and the degree of growth is highly proportional to the gape size of the predators present in a lake. However, the growth coefficient was not correlated to gape size. Indeed, it was inversely related to asymptotic length, probably because of increased time to reach very large maximum body sizes. Maximum life span ranged from 6.1 years in N. Sætertjern to 16.2 years in Bugårdsdammen and was positively correlated with predator gape size.

Predator gape size was negatively correlated with fish density. In predator-free lakes, dense allopatric populations suffer high intraspecific competition and adult mortality, with a few fish reaching large sizes (Tonn et al., 1994). Predation may have an indirect positive effect on prey growth by reducing the density of prey population and releasing survivors from competition (Van Buskirk & Yurewicz, 1998). These dynamics are also expected to be highly dependent on resource availability (Day et al., 2002). Interestingly, in Lakes Posttjernet and Karussputten, crucian carp had the lowest length at age 1 and 3 compared to other lakes with similar fish community. These lakes had the lowest nutrient values, suggesting that growth might be limited by resource availability. Lake structure is particularly important as small lakes at northern latitudes are subject to extreme seasonal changes, such as abrupt rising temperatures during summer and anoxic conditions during winter. Small crucian carp from allopatric populations might invest more energy and time for building up reserves for overwintering compared to large fish from multi-species communities (Vornanen et al., 2011). Moreover, differences in lake structure and abiotic characteristics may explain the great variation in growth traits observed among predator-free populations (Fox & Keast, 1990; Dembski et al., 2006; Tarkan et al., 2011).

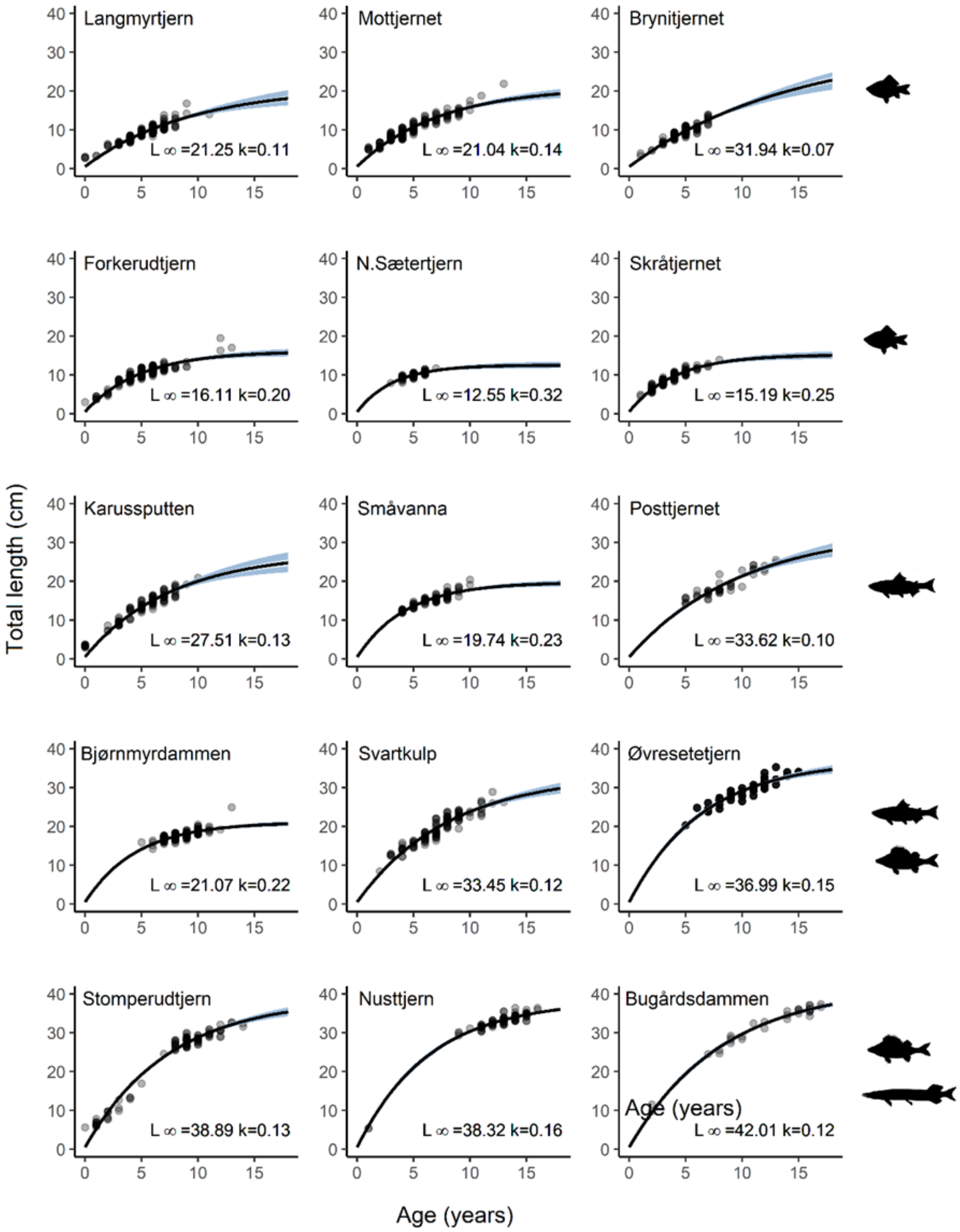

Figure 7. *Von Bertalanffy growth curves for crucian carp from 15 lakes without predator fish (first and second upper rows), with brown trout (second upper row), perch or perch and brown trout (second lower row), and pike and perch (first lower row). Shaded areas around the growth curves correspond to the 95% credibility intervals.*

Reproductive traits

Age at sexual maturity ranged from 2.6 years to 4.0 years, and, contrary to predictions, was not correlated with predation risk. These results were comparable to values of populations from similar latitudes (Holopainen & Pitkänen, 1985), although studies on reproductive traits of crucian carp are rare in Fennoscandia. By contrast, length at maturity was positively correlated with gape size, as a result of increased growth at young age. Gonadosomatic index (GSI) was increasing with predation risk and was positively correlated with reproductive life span. Previous studies found that large females from multispecies communities had higher gonadosomatic index, absolute and relative fecundity, and larger eggs, and proposed that reproductive effort increases with body size (Moisander, 1991; Holopainen et al., 1997b). Moreover, crucian carp is a batch spawner, and the number of spawning events during each season seems to depend on water temperature rather than predation risk or other environmental factors (Aho & Holopainen, 2000). These results seem to contrast with life-history theory since a high reproductive effort should happen at the expense of growth or survival (Stearns, 1992). However, previous studies on crucian carp considered mainly large females that were probably already well outside of the predation window. Small fish from predator lakes may mature early but still invest more energy in growth than reproduction in order to reach a size-refuge from predation. Then, they may slow down growth and allocate more energy to reproduction.

Crucian carp allocate more energy to reproduction, grow older, and attain larger asymptotic sizes in presence of predators, which strongly suggests higher food availability in these lakes. Fish from predator lakes likely experience higher levels of resource availability as an indirect consequence of reduced intraspecific competition caused by predation. High-predation lakes are also highly productive systems that presented a great proportion of complex vegetated littoral habitats. This may favor growth and reproductive allocation even after fish have outgrown predator gape size.

5. General discussion

5.1. A fine-tuned defense mechanism against predation risk

Crucian carp body depth increased along a gradient of predation risk. This change in body shape was accompanied by shifts in population structure and life-history strategies. Increasingly efficient predators determined a decrease in population density, progressively deeper body shape, and larger size (paper I). Moreover, with increasing predation risk, fish grew older, attained larger asymptotic length, and had higher reproductive effort (paper III). Differences in the morphology, biology, and ecology of crucian carp have been traditionally described as a dichotomy between populations from allopatric and multi-species communities where pike is the top predator (Holopainen et al., 1997b). These results show that crucian carp morphology, population structure, and life-history traits range widely between these two extreme morphotypes. Relative body depth was highly proportional to the predation window, strongly suggesting that crucian carp is provided with a fine-tuned morphological defense response to decrease vulnerability to gape-size limited predators (paper I; paper III). This increase in body depth was accompanied by a higher growth rate, especially at young age, further supporting the hypothesis that fish were rapidly achieving a size-refuge to avoid predation (paper III).

As previously observed under experimental conditions (Brönmark & Pettersson, 1994), crucian carp from pike-lakes seem to present the greater response to predation risk, and fish grew well above predator gape size. Pike was coexisting with perch in these lakes, which may have imposed a greater predation risk due to a widening of predation window but also for their different hunting strategies (Eklöv & Diehl, 1994). Crucian carp from perch and/or brown trout lakes presented higher variation in body depth and growth, but always in between allopatric populations and pike-lakes. This was probably associated with the variable dietary preferences of perch and brown trout compared to the strictly piscivorous pike (Mittelbach & Persson, 1998). Thus, this gape-size gradient is also representative of a shift in the species composition and ecology of the predator communities, which may ultimately regulate crucian carp defense response. Crucian carp in small lakes, characterized by frequent fish mortality during winter followed by recolonization of piscivores, have a stochastic environment (e.g. Lappalainen et al., 2016) potentially favoring the evolution of specific inducible antipredator responses. However, regulation of development of body shape in response to predation risk seems to be a complex process and other indirect effects as such resource availability, competition, and behavioral responses seemed to influence crucian carp growth and expression of inducible defense.

5.2. The role of resource use: feeding preferences, competition and lake productivity

Differences in crucian carp body shape were not directly related to predator-induced shifts in resource use (paper I), although we observed an increase in littoral prey preference in presence of predators (paper II). In these highly variable systems, changes in resource availability are likely to occur and plastic feeding strategies might be favored over specialization in acquiring specific resources (Scharnweber et al., 2013). In general, crucian carp experienced better growth conditions along the predation risk gradient, probably favored by the larger resource availability and decreasing intraspecific competition associated with the different predator communities.

We observed a strong correlation between fish density, predator gape size, and fish growth (paper III). Small body sizes, short lifespans, and low reproductive effort suggest that intraspecific competition is a major driver of the ecology of allopatric populations of crucian carp in the wild (Tonn et al., 1994). Size-selective predation could reduce the density of small fish, relaxing intraspecific competition and favoring a subsequent increase in growth of survivors (Persson et al., 1996; Craig et al., 2006). Indeed, crucian carp from populations with absent or low predation risk presented higher individual dietary specialization compared to the other lakes, suggesting that increasing predation risk was releasing intraspecific competition (paper II). Moreover, decreased intraspecific competition coupled to size-related shift in resource use in presence of predators may have also allowed access to a broader range of resources, probably more energetically rewarding compared to small cladocerans or detritus (paper II).

Fish community composition was shifting with lake productivity. In particular, pike-lakes corresponded to the most productive communities, where resources were likely highly available. Large food availability of these lakes may sustain crucian carp growth even in presence of high densities of competitor species (paper II). Interspecific competition, however, may strongly influence crucian carp resource use and growth, and its role is overlooked. Langmyrtjern was the only lake to harbor crucian carp and another no-predator species, minnow. Here, crucian carp had the lowest relative body depth, smallest size, slow growth at young age (paper III), and highest individual dietary specialization (paper II). The combined effect of intra- and interspecific competition might have constrained crucian carp diet breadth, with negative effects on individual growth. On the opposite in Bugårdsdammen, crucian carp reached the highest relative body depth, large sizes, and rapid growth at young age (paper III). This was a productive lake where only pike and perch were present in addition to crucian carp. It could be argued that in absence of other cyprinids, increased predation risk would elicit a stronger antipredator response. Crucian carp from this lake had also the largest isotopic niche area among all lakes (paper II), suggesting increased foraging opportunity compared to other pike-lakes with dense cyprinid communities.

5.3. Patterns of energy allocation under predation risk

Crucian carp development in body depth seems to be the result of overall better growth conditions associated with larger resource availability with increasing predation risk, rather than a defense directly induced by the predator presence. However, if this theory is true, how can we explain crucian carp increase in body depth after simple exposure to predation cues? Activity, growth, and morphology are often highly integrated traits and therefore changes in activity levels affecting energy acquisition can, in turn, affect morphology (Pigliucci, 2005). Experiments show that crucian carp exposed to predation cues respond with a lowering of activity levels (Holopainen et al., 1997a). This reduction in activity may save energy, which is then allocated to somatic growth, determining body development (Johansson & Andersson, 2009). Crucian carp with a body height below predator gape size had very low catchability in this and other field studies (Brönmark et al., 1995; Vornanen et al., 2011), suggesting that small individuals vulnerable to predation may have very low activity levels. Still, small fish allocated energy to growth and reproduction (paper III). Laboratory experiments show that crucian carp habitat use was significantly affected by both predation risk and hunger level, indicating a trade-off between food acquisition and predator avoidance (Pettersson & Brönmark, 1993). In the wild, the structural complexity of vegetated littoral habitats in high-predation lakes may offer fish food and shelter until they reach a size-refuge. Here, a deep body might also provide fish with greater maneuverability and foraging efficiency to feed on benthic invertebrates associated with the substrate or vegetation (Svanbäck & Eklöv, 2003). Moreover, crucian carp can alter diel activity patterns when occurring with predators (Vinterstare et al., 2020). Increased habitat complexity, food availability and high plasticity in diet may allow fish to get enough energy for allocation to growth and reproduction while lowering foraging activity. By contrast, high competition for resources in absence of predators, likely forces individuals to increase foraging activity to avoid starvation, resulting in stunted growth and low reproductive effort. Fish from allopatric populations may also invest more energy in building up winter reserves (Vornanen et al., 2011). A deep-bodied shape, however, resulted costly when competing for limited resources in absence of predators (Webb 1984; Pettersson & Brönmark, 1997).

6. Conclusion

Crucian carp variation in body shape represents a fine-tuned defense response against increasingly efficient gape-limited predators. Variation in body depth was accompanied by a clear shift in population structure and life-history strategies. Reduced intraspecific competition, larger food availability, and habitat complexity likely favored energy acquisition despite potential low activity levels associated with predation risk. Plastic feeding habits and a shift in resource use with increasing body size may also have supported this energy-saving mechanism. The energy saved from reduced foraging activity was then allocated to both growth and reproduction. Predation is a major selective force favoring the evolution of inducible defenses, however, the observed anti-predator response in crucian carp was likely a result of evolutionary optimization of predation risk with the prevailing environmental conditions. In this work, I related the crucian carp antipredator defense response to a broader environmental and ecological context, offering new insights on how selection can maintain predator-induced plasticity. This may contribute to a better understanding of the evolutionary and ecological significance of phenotypic plasticity in small lakes with high environmental stochasticity. Environmental variation driving plastic changes in one organism may have effects that spread throughout the ecosystems. The understanding of these dynamics is particularly important nowadays since small freshwater ecosystems are particularly exposed to anthropogenic pressure.

7. Caveats and future directions

In this field study, predator community was shifting along a productivity gradient. On one side, this allowed us to observe how different selective forces operate on several traits in complex, real-world situations. On the other side, there were limitations in distinguishing the effect of predation risk from resource availability. Moreover, the smallest size classes were often underrepresented in high-predation lakes. Future studies should investigate the changes associated with the predator-induced defense along a gradient of resource availability in controlled experimental settings. Changes in body shape, activity levels, metabolism, and investment in growth and reproduction could be quantified to better understand the patterns of energy allocation of crucian carp.

Crucian carp from allopatric populations had different relative body depth and highly variable life-history traits. These fish can inhabit very heterogeneous systems and future investigations should examine how different environmental conditions influence crucian carp population dynamics and energy allocation in absence of predators.

In this study, both intra- and interspecific competition seemed to play an important role in crucian carp diet preferences and growth. In order to fully address the effect of resource competition, future studies should investigate crucian carp resource use and growth under different exposures to competitor species in pond or mesocosm experiments.

Laboratory and field experiments often assume that the development of an inducible defense in crucian carp is exclusively due to a plastic response. However, natural population of crucian carp may present differences in the degree of expression of the inducible defense as a result of selection. Future studies should investigate if there is a genomic component associated with the phenotypic expression. Common garden experiments could be also used to compare the expression of the inducible defense among fish from locations with different historical exposure to predators.

Future studies investigating crucian carp resource use should include data on prey abundance for estimation of pelagic and littoral habitat profitability. Gut contents analysis gives a snapshot of fish prey preferences and may not reflect fish optimal diet choices. Stable isotopes are particularly useful in complementing gut contents analysis as they can give information on resource use over a longer time frame. However, they do not give taxonomical resolution. Compound-specific stable isotopes could be used as a complementary methodology to get a better resolution of crucian carp resource use.

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Dissertation articles

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ORIGINAL RESEARCH

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Predator community and resource use jointly modulate the inducible defense response in body height of crucian carp

Ilaria de Meo[1] | Kjartan Østbye[1] | Kimmo K. Kahilainen[2] | Brian Hayden[3] | Christian H. H. Fagertun[1] | Antonio B. S. Poléo[1]

[1]Department of Forestry and Wildlife Management, Inland Norway University of Applied Sciences, Koppang, Norway

[2]Lammi Biological Station, University of Helsinki, Helsinki, Finland

[3]Biology Department, Canadian Rivers Institute, University of New Brunswick, Fredericton, NB, Canada

Correspondence
Ilaria de Meo, Department of Forestry and Wildlife Management, Inland Norway University of Applied Sciences, Campus Evenstad, Koppang, Norway.
Email: ilaria.demeo@inn.no

Funding information
Inland Norway University of Applied Sciences

Abstract

Phenotypic plasticity can be expressed as changes in body shape in response to environmental variability. Crucian carp (*Carassius carassius*), a widespread cyprinid, displays remarkable plasticity in body morphology and increases body depth when exposed to cues from predators, suggesting the triggering of an antipredator defense mechanism. However, these morphological changes could also be related to resource use and foraging behavior, as an indirect effect of predator presence. In order to determine whether phenotypic plasticity in crucian carp is driven by a direct or indirect response to predation threat, we compared twelve fish communities inhabiting small lakes in southeast Norway grouped by four categories of predation regimes: no predator fish, or brown trout (*Salmo trutta*), perch (*Perca fluviatilis*), or pike (*Esox lucius*) as main piscivores. We predicted the body shape of crucian carp to be associated with the species composition of predator communities and that the presence of efficient piscivores would result in a deeper body shape. We use stable isotope analyses to test whether this variation in body shape was related to a shift in individual resource use—that is, littoral rather than pelagic resource use would favor the development of a specific body shape—or other environmental characteristics. The results showed that increasingly efficient predator communities induced progressively deeper body shape, larger body size, and lower population densities. Predator maximum gape size and individual trophic position were the best variables explaining crucian carp variation in body depth among predation categories, while littoral resource use did not have a clear effect. The gradient in predation pressure also corresponded to a shift in lake productivity. These results indicate that crucian carp have a fine-tuned morphological defense mechanism against predation risk, triggered by the combined effect of predator presence and resource availability.

KEYWORDS
body shape, lake productivity, phenotypic plasticity, predation risk regime, resource use

1 | INTRODUCTION

Phenotypic plasticity is the ability of an organism to express different phenotypes in response to environmental variation (Pigliucci, 2001). Plastic responses can be a successful strategy in spatially or temporally heterogeneous environments, where organisms can improve their fitness by adjusting morphological, physiological or behavioral traits in relation to different abiotic and biotic conditions (Gabriel, 2005; Lind & Johansson, 2007; Miner et al., 2005). However, the ecological and evolutionary significance of phenotypic plasticity is still under debate (Pfennig et al., 2010; Price et al., 2003; Uller et al., 2019). Phenotypic plasticity can facilitate adaptation to novel environments, allowing populations to occupy different ecological niches that may lead to speciation events (Corl et al., 2018; Skúlason et al., 2019). At the same time, its benefits could be constrained by the energetic costs associated with the production and maintenance of plastic responses as well as limits in the predictability and reliability of environmental cues (DeWitt et al., 1998; Snell-Rood et al., 2010).

Phenotypic plasticity can be expressed as variation in body shape in response to interactions with other species, different resource availability, as well as different habitat characteristics. Many freshwater organisms can adopt predator-induced morphological defenses when exposed to a predation threat (Bourdeau & Johansson, 2012; DeWitt et al., 2000; Sperfeld et al., 2020). Here, chemical cues from predators or injured conspecifics induce a morphological change in the prey that make them less vulnerable to predation (Harvell, 1990). For example, in the presence of predators, pumpkinseed sunfish (*Lepomis gibbosus*) increases its defensive structure such as dorsal spine length and body depth (Januszkiewicz & Robinson, 2007). Another classic example of inducible antipredator defense mechanism in fish is the crucian carp (*Carassius carassius*), which develops a deep body when exposed to cues from predators such as perch (*Perca fluviatilis*) or pike (*Esox lucius*) (Brönmark & Pettersson, 1994). Flexibility in prey morphological and behavioral responses might be a widespread strategy, given that species composition of predators often varies greatly among locations and over time (Kishida & Nishimura, 2006). Indeed, although with consistent differences among species, variation in body shape in response to predator presence has been hypothesized in various freshwater fish such as perch, roach (*Rutilus rutilus*), three-spined sticklebacks (*Gasterosteus aculeatus*), and fathead minnow (*Pimephales promelas*) (Eklöv & Jonsson, 2007; Frommen et al., 2011; Meuthen et al., 2019).

In general, predators play an important role in structuring freshwater ecosystems. Different predators can influence prey dynamics and select specific morphological and behavioral traits of prey by variation in their density, gape size and foraging strategies (Magnhagen & Heibo, 2004; Scharf et al., 2000; Sharma & Borgstrøm, 2008). For example, pike is a sit-and-wait predator, attacking from littoral vegetation (Skov & Nilsson, 2018; Turesson & Brönmark, 2004) and tends to prey upon nonvigilant individuals (Heynen et al., 2017). In contrast, piscivorous perch hunt actively for prey and select mainly mobile, bold individuals (Heynen et al., 2017). Piscivorous fish such as pike are also gape-size limited in their prey selection and often prefer to select shallow-bodied individuals, since handling time increases with prey body depth (Nilsson et al., 1995). Selective consumption can cause a shift in the phenotypic distribution of prey, since large deep-bodied individuals which are outside the predation window are more likely to survive (Nilsson & Brönmark, 2000). In turn, this shift can have indirect effects that influence dramatically prey competitive interactions and community dynamics (Peacor & Werner, 2001; Siepielski et al., 2020).

Moreover, the role of resource use in predator-induced morphological defenses has been recently debated, since trade-offs occur among predation risk and resource acquisition (Scharnweber et al., 2013; Svanbäck et al., 2017). In this sense, lake morphology and water quality regulate availability and quality of food resources that, in turn, influence both population density and individual growth rate (Horppila et al., 2010). In particular, fish condition generally increases with lake productivity since nutrients fuel the base of the food web, increasing available resources for consumers (Weber et al., 2010). At the same time, food acquisition and growth rate are often highly influenced by intraspecific competition and thus negatively related to population density (Amundsen et al., 2007; Svanbäck & Persson, 2004). Predation can also indirectly induce a change in prey morphology causing shifts to habitat with different food quality (Preisser et al., 2005). In this case, an alteration in prey phenotype can represent a foraging adaptation that promotes specialization in acquiring specific resources in the new habitat (Ellerby & Gerry, 2011). Thus, it is pertinent to address the question whether the predator cues alone result in morphological change in the prey, or if the changed foraging habitat of the prey is driving the altered morphology as a secondary response. Alternatively, and more likely, evolutionary optimization of the trade-off regime may result from both selective pressures jointly.

In this study, we examine crucian carp body shape and depth from lakes and ponds with different piscivore assemblages and environmental characteristics, testing also for associations between predator-induced shifts in resource use and morphology. Because of its unique physiological adaptations, crucian carp is often the only fish species able to survive in anoxic waters of shallow ponds during winter (Blažka, 1958; Piironen & Holopainen, 1986). High densities of small-sized and shallow-bodied fish characterize populations occurring in these ponds, where obvious resource limitation leads to strong intraspecific competition (Pettersson & Brönmark, 1997). On the other hand, multispecies assemblages in larger lakes contain low densities of deep-bodied crucian carp, for which predation is likely the main regulating force (Poléo et al., 1995). In these lakes, a deep body represents a morphological defense against gape-limited predators (Nilsson & Brönmark, 2000). However, experiments have shown that enhanced food availability and low population densities alone can cause a similar increase in relative body depth compared to predation risk (Holopainen et al., 1997; Tonn et al., 1994), suggesting that growth and morphology are also dependent on resource availability. Moreover, in a manipulative experiment, Andersson et al., (2006) observed that crucian carp feeding on benthic prey rather than zooplankton developed a deeper body, similarly to the fish exposed to cues from predators. With an analogous experimental approach, it was found that both standing water conditions and exposure to predation cues independently induced a deeper body in crucian carp (Johansson & Andersson, 2009). Thus, it has been proposed that this increase in body depth could be associated with an alteration in foraging behavior and activity levels of the fish, suggesting that more complex mechanisms may control the morphology of this species than the sole predation risk (Vøllestad et al., 2004). Laboratory experiments also show that crucian carp habitat use was significantly affected by both predation risk and hunger level, indicating a trade-off between food acquisition and predator avoidance (Pettersson & Brönmark, 1993). Moreover, in presence of predators, the structural complexity offered by vegetation of near shore habitats may enhance the chance of survival of crucian carp until they reach a certain body depth (Holopainen et al., 1997). In this environment, benthic invertebrates associated with the substrate or vegetation are the most abundant prey type and a deep body might provide fish with a greater maneuverability and foraging efficiency (Svanbäck & Eklöv, 2003; Webb, 1984). In contrast, if predation pressure is released, fish would rely more on pelagic invertebrates and show a slender body shape. Hence, discerning how different environmental factors affect plastic responses in crucian carp may help us gain a better understanding of their evolutionary and ecological significance for freshwater fish.

Here, we examined crucian carp body shape and trophic niche variability by landmark-based geometric morphometrics and stable isotope analysis (SIA) in a series of small lakes. We used stable isotopes of carbon and nitrogen to estimate the trophic position and relative contribution of littoral and pelagic energy to each crucian carp sampled in each lake. Individual trophic specialization could reveal potential variation in crucian carp habitat preferences and resource use, which could be reflected in specific body morphology. Thus, a more extensive morphometric analysis of wild populations of this species could potentially show variation in different traits other than body depth. This approach differs from previous experimental studies (Andersson et al., 2006; Johansson & Andersson, 2009; Vøllestad et al., 2004), as we consider a comprehensive set of abiotic and biotic characteristics that might directly or indirectly underlie crucian carp body development. Moreover, we included locations with multiple predators to quantify the predation risk associated with each lake. Here, a set of three replicate lakes of four categories of predation regimes was tested, being allopatric lakes (no predators), and sympatric lakes with three increasingly efficient main predators: brown trout (*Salmo trutta*), perch, and pike. Brown trout and perch are opportunistic piscivores. In small lakes, invertebrates are the major food source of brown trout and perch until they shift to a diet mainly consisting of fish, and this switch to piscivory generally occurs at a larger size for trout (Mittelbach & Persson, 1998). On the contrary, pike is a specialist piscivore throughout its life and grows to large body and gape size, representing an efficient predator that can impose a greater risk for a broad range of prey size classes (Mittelbach & Persson, 1998).

In particular, in the present study, we expected the body shape of crucian carp to be associated with the species composition of predators in the lakes investigated and thus that increasingly efficient predators would cause progressively deeper body shape. We also wanted to evaluate if such variation in body shape depended on predator-induced shifts in habitat and resource use. Specifically, we wanted to test whether crucian carp under increasing predation risk feed more on littoral resources associated with substrate or vegetation, compared to crucian carp in lakes without predators (Pettersson & Brönmark, 1993). Moreover, we predicted that variation in body shape was modulated by the synergistic effect of predation risk (predator mouth gape and density) and specific abiotic factors (lake morphology and productivity).

2 | METHODS

2.1 | Study area and sample collection

We sampled twelve fish communities from small (0.25–11 ha) and relatively shallow (max depth 1.5–11.3 m) lakes located in southeastern Norway between June and August in 2018 and 2019 (Figure 1; Table 1). All lakes were characterized by high densities of macrophytes. Abiotic parameters collected include lake surface area (ha), maximum depth (m), and nutrient concentration (Table 1). We estimated lake bathymetry in the field with a portable echosounder. Total nitrogen (μg/l), phosphorus (μg/l), and organic carbon (mg/l) were determined from surface water samples for ten lakes and retrieved from the Norwegian Environment Agency for two lakes (vannmiljofaktaark.miljodirektoratet.no).

Locations were chosen along a gradient of predation pressure. We grouped lakes into four categories according to species composition of predators in the systems: no predators, brown trout (hereafter trout), perch, and pike lakes (Tables 2 and 3). No predators occurred in Forkerudtjern, Langmyrtjern, and Motjennet. Brown trout was the only predator in Karussputten, Småvanna, and Posttjernet. Perch was the main predator in Svartkulp, Bjørnmyradammen, and Øvreseterjern while trout was present with very low density or absent. Both perch and pike were present in Bugårdsdammen, Stomperudtjernet, and Nusttjennet, but we will refer to these lakes as "pike lakes" for simplicity. We assessed fish density in each lake using Nordic multimesh gillnets consisting of 12 equidistant panels (mesh sizes 5–55 mm) and calculated CPUE (*n* fish·net^{-1} h^{-1}) for littoral, profundal, and pelagic habitats. Then, proportions of predators and crucian carp were calculated as the respective densities from CPUE data relative to the total fish present in each lake (Table 3). Some lakes had very limited pelagic and profundal habitats and were considered as entirely littoral. Moreover, we did not catch any fish in the profundal zone, probably because deeper lakes were highly humic systems with hypoxic deep waters. Consequently, fish density and biomass analysis were limited to the littoral and pelagic zones. We also used a variety of fishing methods (e.g., baited traps, gillnets with different mesh sizes, kick nets) to increase our catch of small crucian carp, since these fish often display an elusive behavior and alter

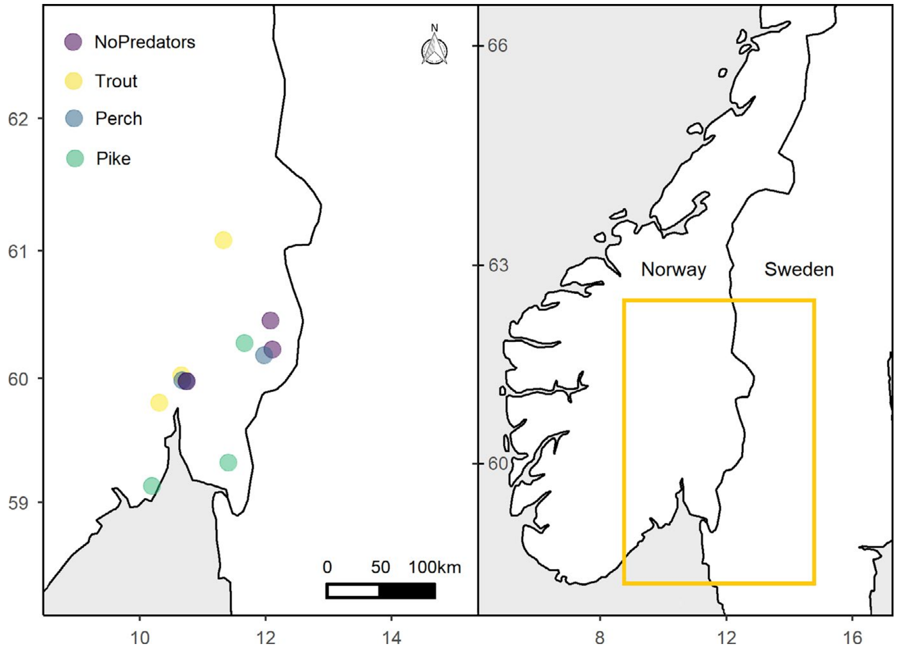

FIGURE 1 Location of the twelve sampling sites in southeastern Norway

TABLE 1 Environmental characteristics of the study lakes

Lake	Lat (°N)	Long (°E)	Alt (m a.s.l.)	Area (ha)	MaxD (m)	TotN (µg/l)	TotP (µg/l)	TOC (mg/l)	Fish species
Bugårdsdammen	59.13	10.2	42	5.04	2	980	54	9.5	a, b, c
Stomperudtjernet	59.32	11.4	103	3.85	1.5	1,660	146	18.4	a, b, c, e, f, g
Nusttjennet	60.28	11.66	131	11.00	1.5	1,090	164	16.4	a, b, c, e, f
Øvresetertjern	59.98	10.67	478	3.05	3.5	446	13	6.6	a, c, d
Svartkulp	59.98	10.74	202	5.80	10	550	13	9.9	a, c, d, h
Bjørnmyrdammen	60.18	11.98	256	2.10	3.5	672	26	6.5	a, c, i
Posttjernet	61.08	11.33	271	1.72	11	312	8	9.7	a, d, h
Småvanna	59.8	10.31	222	0.50	3.8	616	14	10.1	a, d, h
Karussputten	60.02	10.66	356	0.25	4.6	361	9	5.4	a, d
Forkerudstjennet	60.45	12.08	152	1.24	2.2	1,985	82	23.4	a
Langmyrtjern	59.97	10.75	206	0.30	5	702	20	14.2	a, h
Motjennet	60.23	12.11	167	0.94	11.3	688	23	11.2	a

Note: Variables include latitude (Lat), longitude (Long), altitude (Alt), lake area (Area), maximum depth (MaxD), total nitrogen (TotN), total phosphorus (TotP), total organic carbon (TOC), and fish species present.

Fish species: (a) crucian carp; (b) pike; (c) perch; (d) brown trout; (e) roach *Rutilus rutilus*; (f) bream *Abramis brama*; (g) rudd *Scardinius erythrophthalmus*; (h) minnow *Phoxinus phoxinus*; (i) tench *Tinca tinca*.

diel activity patterns when occurring with predators (Vinterstare et al., 2020). Immediately after capture, fish were euthanized by an overdose of tricainemethanesulfonate (MS222) and transported to the laboratory. Permission to catch fish was given by the Norwegian Environmental Agency (2018/4155) and fish were sampled after oral approval by the local landowners.

In order to estimate the basal resources for stable isotope analysis (SIA), we collected qualitative samples of benthic invertebrates and zooplankton. We sampled benthic invertebrates from sediments and plants in the littoral habitat using kick nets and sorted them to the lowest feasible taxonomic level. We collected zooplankton from several hauls through the water column in the pelagic zone of deep lakes and in the nonvegetated area of shallow lakes with a 50-µm mesh plankton net. Samples were later sieved through a 200-µm mesh to remove unwanted material. The remaining zooplankton were identified to class level.

TABLE 2 Mean and standard deviation of total length (TL), body height (BH), carbon (δ13C) and nitrogen (δ15N) stable isotopes, littoral reliance (LIT), trophic position (TP), and sex ratio of crucian carp

Lake	Predation category	TL (cm)	BH (cm)	δ^{13}C (‰)	δ^{15}N (‰)	LIT	TP	Sex ratio (m/f)
Bugårdsdammen	Pike	31.5 ± 6.8	12.4 ± 2.4	−30.2 ± 0.7	8.7 ± 0.7	0.5 ± 0.3	1.9 ± 0.2	6.5
Stomperudtjernet	Pike	19.1 ± 9.5	7.7 ± 3.9	−32.2 ± 0.7	13.6 ± 1.2	0.9 ± 0.4	1.9 ± 0.3	4
Nusttjennet	Pike	33.1 ± 1.6	13.5 ± 0.5	−31.6 ± 0.3	10.6 ± 0.4	0.8 ± 0.0	1.8 ± 0.1	1.3
Øvresetertjern	Perch	28.8 ± 3.6	10.6 ± 1.4	−28.1 ± 0.5	7.0 ± 0.4	0.8 ± 0.4	2.2 ± 0.1	1.3
Svartkulp	Perch	19.8 ± 4.7	6.5 ± 1.6	−32.3 ± 0.7	5.6 ± 0.4	0.9 ± 0.2	2.0 ± 0.1	2
Bjørnmyrdammen	Perch	18.0 ± 1.7	6.0 ± 06	−32.2 ± 0.8	6.0 ± 0.4	0.6 ± 0.1	2.3 ± 0.1	1.5
Posttjernet	Trout	19.3 ± 3.6	6.9 ± 1.5	−34.1 ± 1.3	5.7 ± 0.3	0.5 ± 0.3	2.3 ± 0.1	1.7
Småvanna	Trout	15.4 ± 2.2	4.9 ± 0.7	−36.5 ± 1.4	8.6 ± 0.7	0.4 ± 0.3	2.0 ± 0.2	1.4
Karussputten	Trout	14.5 ± 3.4	4.5 ± 1.1	−35.0 ± 1.2	3.6 ± 0.4	0.1 ± 0.3	1.5 ± 0.1	1
Forkerudstjennet	No pred.	11.4 ± 1.9	3.3 ± 0.7	−31.9 ± 1.0	10.1 ± 0.9	0.8 ± 0.2	2.2 ± 0.2	0.2
Langmyrtjern	No pred.	10.7 ± 2.8	2.8 ± 0.9	−34.5 ± 0.9	4.5 ± 0.6	0.5 ± 0.3	1.7 ± 0.2	0.3
Motjennet	No pred.	11.9 ± 3.0	3.4 ± 0.9	−32.7 ± 1.4	5.4 ± 0.7	0.6 ± 0.2	2.1 ± 0.2	1

TABLE 3 Predator species present in each lake, number of pike, perch and trout measured in the laboratory, mean and standard deviation of maximum predator gape size (MaxGS, *n* = 10), predator density (CPUEpred) and proportion (RelPred), and crucian carp density (CPUEcc) and proportion (RelCc)

Lake	Predator species	No. Pike	No. Perch	No. Trout	MaxGS (mm)	CPUEpred (n.net^{-1} h^{-1})	RelPred (%)	CPUEcc (n.net^{-1} h^{-1})	RelCc (%)
Bugårdsdammen	Pike, perch	27	337	–	61.0 ± 14.2	1.6 ± 0.6	93.8	0.1 ± 0.1	6.2
Stomperudtjernet	Pike, perch	7	25	–	56.2 ± 26.0	0.4 ± 0.0	5.0	0.4 ± 0.2	5.7
Nusttjennet	Pike, perch	27	24	–	47.3 ± 11.9	0.3 ± 0.3	4.6	0.4 ± 0.2	6.2
Øvresetertjern	Perch, trout	–	286	36	44.3 ± 7.0	3.6 ± 1.9	89.4	0.4 ± 0.6	10.6
Svartkulp	Perch, trout	–	151	7	34.8 ± 4.4	2.1 ± 1.3	68.9	0.4 ± 0.4	12.2
Bjørnmyrdammen	Perch	–	34	–	26.7 ± 2.1	0.4 ± 0.3	16.1	2.1 ± 2.1	83.2
Posttjernet	Trout	–	–	89	37.9 ± 1.7	0.9 ± 1.1	53.3	0.2 ± 0.2	10.6
Småvanna	Trout	–	–	17	33.1 ± 7.0	0.3 ± 0.4	18.0	1.0 ± 1.1	60.8
Karussputten	Trout	–	–	12	32.8 ± 4.8	0.2 ± 0.2	13.8	1.0 ± 0.2	86.2
Forkerudstjennet	–	–	–	–	–	–	–	10.6 ± 5.9	100.0
Langmyrtjern	–	–	–	–	–	–	–	2.1 ± 1.8	77.0
Motjennet	–	–	–	–	–	–	–	7.8 ± 4.4	100.0

2.2 | Laboratory analysis

Body shape was measured from a total of 360 crucian carp. From each lake, we subsampled 30 crucian carp for morphometric analysis (Table 2). In general, fish size structure was quite uniform within each lake and dominated by large individuals. Thus, we included smaller crucian carp whenever possible to avoid underrepresentation of this size class. From the same fish, a piece of dorsal muscle tissue was dissected and frozen at −20°C for SIA. We also measured length and gape height of the most abundant piscivorous fish, that is, trout, perch, and pike (Table 3). Mouth height was measured as the maximum distance between the tip of the premaxilla and the mandible with the mouth stretched open. Then, we calculated the mean maximum gape size from each lake by selecting the predators with the highest mouth height, irrespective of the species (*n* = 10).

2.2.1 | Morphometric analysis

We examined crucian carp body shape using landmark-based geometric morphometrics. We laterally photographed fish using a Nikon D5300 camera positioned on a tripod and set at a focal length of around 60 mm. In order to minimize perspective and distortions errors among images, we arranged fish along their main horizontal axis, extended dorsal and ventral fins using dissecting pins, and used a mesh cradle (Muir et al., 2012). Digital photographs were transferred to tpsDig2 software v 2.31 (Rohlf, 2004), and 17 landmarks and six semilandmarks were digitized (Figure 2).

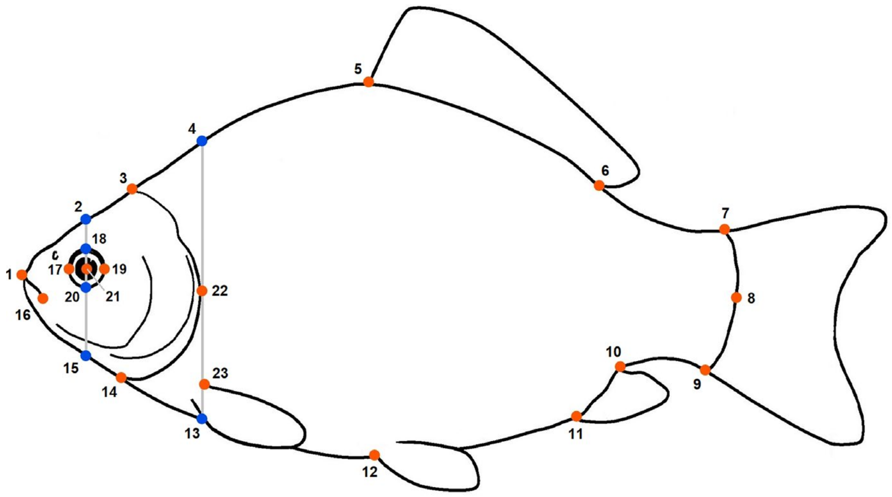

FIGURE 2 Crucian carp line drawing showing the location of 17 landmarks (red dots) and 6 semilandmarks (blue dots) used in geometric morphometric analysis. Homologous landmarks (red dots) indicate tip of the snout (1), posterior dorsal margin of the head (3), anterior insertion of dorsal fin (5), posterior insertion of dorsal fin (6), dorsal insertion of caudal fin (7), posterior margin of caudal peduncle (8), ventral insertion of caudal fin (9), posterior insertion of caudal fin (10), anterior insertion of caudal fin (11), insertion of pelvic fin (12), posterior ventral margin of the head (14), posterior margin of mouth (16), anterior margin of eye (17), posterior margin of eye (19), center of eye (21), posterior margin of operculum (22), dorsal insertion of pectoral fin (23). Semilandmarks placed along axis passing through the center of eye and the posterior edge of the operculum indicate dorsal midpoint of head (2), anterior dorsal midpoint of body (4), anterior ventral midpoint of body (13), ventral midpoint of head (15), dorsal margin of eye (18), and ventral margin of eye (20). Picture adapted from the Crucian Carp Field Identification Guide by the UK Environment Agency (www.gov.uk/environment-agency)

Digitizing was always performed by the same person. After checking for outliers, we used a Generalized Procrustes Analysis (GPA) to standardize the landmark configurations for position, orientation, and size. Centroid size (CS) of the landmark configurations was used as a proxy for body size. Centroid size is the square root of the sum of the squared distances of landmarks from their center of gravity (centroid). Centroid size values were log-transformed prior to statistical analysis. All morphometric analysis was performed using the package “Geomorph” (Adams et al., 2020) in R version 4.0.1 (R Core Team, 2020).

2.2.2 | Stable isotope analysis

Fish muscle and invertebrate samples were freeze-dried at −50°C for 48 hr, ground to a homogeneous powder, weighed (1.0–1.2 mg), and encapsulated into tin cups. Stable carbon and nitrogen isotope ratios were analyzed by a Costech 4010 elemental analyzer (Costech) coupled to a Delta Plus continuous flow mass spectrometer (Thermo Finnigan). Stable isotope measurements are expressed as δ^{13}C and δ^{15}N in parts per thousand (‰) relative to the international standards Vienna Pee Dee Belemnite and atmospheric air for carbon and nitrogen, respectively. Standard deviation of internal working standards was less than 0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N. C:N ratios from fish data were low in all samples (3.3 ± 0.1) indicating low lipid concentrations (Fagan et al., 2011; Kiljunen et al., 2006). Thus, we did not lipid-correct δ^{13}C ratios. Since basal resource values can vary greatly among different systems, we standardized crucian carp δ^{13}C and δ^{15}N ratios by using littoral and pelagic invertebrates in each lake as baseline. Individual trophic position and littoral reliance (i.e., relative contribution of littoral prey items to crucian carp diet) were calculated using a two-source mixing model (Karlsson & Byström, 2005) with trophic fractionation values of 3.4‰ for δ^{15}N and 0.4‰ for δ^{13}C (Post, 2002).

2.3 | Data analyses

2.3.1 | Body shape analysis

Principal component analysis (PCA) on Procrustes shape coordinates was used to identify the major patterns of shape variation and grouping of variance among individuals. Thin-plate deformation grids were used to visualize variation at the lowest and highest values along the first principal component axis. In order to investigate variation of crucian carp body shape among predation categories (no predators, trout, perch, and pike), principal component scores were examined through Discriminant Function Analysis (DFA) in the R package “MASS.” The maximum number of principal components to retain in the analysis was estimated by the broken stick model. Validity of discrimination was tested by jackknifed cross-validation. A Procrustes ANOVA with permutation procedures was used to estimate allometric effects (i.e., shape variation in relation to size) among predation categories in the R package “Geomorph” (Adams et al., 2020). Procrustes shape coordinates were used as response variables, log-transformed centroid size as predictor variable and predation as categorical variable with lake as nested effect. Since allometry had a significant effect on shape, centroid size was used as a covariate in subsequent linear models. Shape differences between sexes were significantly different but explained only a very small part of variation (R^2: 0.047, *p*-value: 0.001). Males had slightly larger dorsal region than females; however, females alone expressed the same changes along the PC axes, indicating a minor effect of sex. Therefore, females and males were pooled in the analysis.

2.3.2 | Association of body depth with environmental variables

We determined if crucian carp assemblages occupied distinct isotopic niches using a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) of a Euclidean distance matrix of littoral reliance and trophic position. Predation category and Lake were used as factors in the analysis. In addition, we used a distance-based test for homogeneity of multivariate dispersions (PERMDISP; Anderson, 2006) to evaluate differences in within-group variability of Lakes and Predation factors. Analysis was performed in R using the *adonis* and *betadisper* functions in the “vegan” package (Oksanen et al., 2019). We used linear mixed-effects models (LME) to examine the degree of relationship between variation in body depth and specific biotic and abiotic characteristics associated with each lake. The scores of the first axis of principal component (PC1), which corresponded largely to the fish body depth, were used as the response variable. More precisely, considering that Procrustes superimposition controls the size effects through scaling, the response variable represents crucian carp relative body depth. Candidate explanatory variables for predation risk included predation category (Pred), maximum predator gape size (MaxGS), predator density (CPUEPred), and predator proportion (RelPred). Density (CPUECc) and proportion (RelCc) of crucian carp were used as a proxy for intraspecific competition. However, predator density and proportion were positively correlated ($r > 0.8$), and only the latter was included in the final model. Moreover, both crucian carp density and proportion were excluded, since negatively correlated with the maximum predator gape size ($r < -0.8$). Littoral reliance (LIT) and trophic position (TP) were used as a measure of individual crucian carp resource use. Abiotic characteristics included lake area, maximum depth (MaxD), and total nutrients. Among nutrient variables, only total phosphorus (TotP) was used in the analysis, since it was positively correlated with both total nitrogen and organic carbon ($r \geq 0.7$). The full model takes the form:

$$\text{Body depth} \sim \text{RelPred} + \text{MaxGS} + \text{LIT} + \text{TP} + \text{MaxD} + \text{TotP} + \text{Area} + \text{logCsize}.$$

Model selection was performed by stepwise selection based on the Akaike information criterion (AIC). Lakes were used as a random factor nested in the predation category term. Model assumptions of normality and homogeneity of residuals were met and validated using a QQ-plot and plotting residuals against fitted values, respectively. Correlation between variables was tested using the *ggpairs* function in the “GGally” package (Emerson et al., 2013). Analyses were performed in R using “lme4” and “lmerTest” packages (Bates et al., 2015).

3 | RESULTS

3.1 | Body shape analysis

The first three axes of the PCA of landmark configurations (Figure 3) accounted for 67% of the variation in body shape, with PC1, PC2, and PC3 explaining 45%, 15%, and 7% of the total variance, respectively. Shape variation along the PC1 axis was mainly associated with the expansion of the dorsal (landmarks 4, 5, 6) and ventral (landmarks 11, 12) regions, indicating an overall change in body depth (see Table S1 in Appendix S1). PC2 described mainly variation in body curvature, with snout (landmarks 1, 16) and caudal peduncle (landmarks 6, 7, 8) bending slightly downwards, and ventral and dorsal parts (landmarks 4, 5, 12) shifting upwards, indicating an overall flattering of the ventral region along the axis. PC3 explained variation in head size (landmarks 1, 22), body slenderness (landmarks 4, 5, 7, 8, 11, 12, 13), and insertion of the pectoral fin (landmark 23). Fish grouped along the first PC axis mainly according to the different predation categories. In absence of predators, fish had a slender body shape, which got increasingly rounded in presence of trout, perch, and pike. The same grouping was revealed by DFA as complementary method (Figure S1). DFA produced three significant DF axes differentiating between predation categories, and DFA1, DFA2, and DFA3

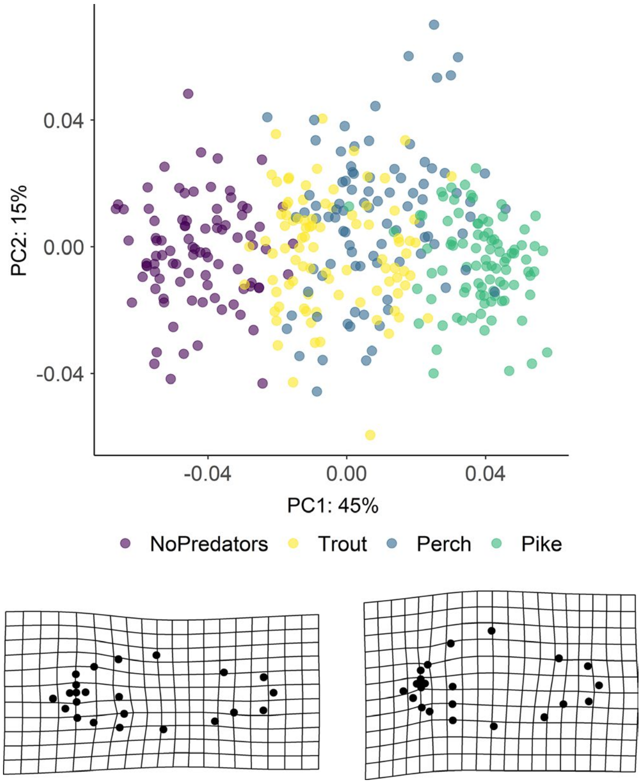

FIGURE 3 Scatterplot of principal components analysis of body shape of crucian carp. Individuals are color-coded according to predation category. Deformation grids show the most extreme negative and positive shapes along the first (PC1) axis. Percentages indicate how much of the variation is explained by the first two axes

accounted for 92.7%, 7.2%, and 0.1% of shape variation, respectively. Jackknifed validation indicated that 79% of crucian carp were assigned to the correct predation category (Table S2). Individuals from "No predators" and "Pike" groups were generally correctly classified (≥90%), while individuals from "Trout" and "Perch" groups were more frequently classified as each other. Results of Procrustes ANOVA (Table S3) show that the body shape of crucian carp was positively related to the logarithm of centroid size (*F*: 191.42, *p*-value: 0.001), predation category (*F*: 46.41, *p*-value: 0.001) and their interaction with (*F*: 6.89, *p*-value: 0.001) and without lake effect (*F*: 7.19, *p*-value: 0.001). The significance of the interaction terms suggests nonparallel slopes and thus that shape variation in relation to size differs among predation categories—for example, small-sized fish from pike lakes have deeper body depth than fish of the same size from lakes without predators (Figure 4).

3.2 | Major variables explaining variation in body depth

The range of littoral reliance and trophic position values observed in each population was significantly different among predation categories (PERMANOVA, Pseudo-*F*: 27.35, *p*-value: 0.001) and lakes (PERMANOVA, Pseudo-*F*: 42.67, *p*-value: 0.001). However, significant differences may be caused by different dispersion of isotopic values for both predation categories (PERMIDISP, Pseudo-*F*: 10.45, *p*-value: 0.001) and lakes (PERMIDISP, Pseudo-*F*: 9.23, *p*-value: 0.001), suggesting great variation in individual resource use within assemblages. Results of model selection for crucian carp body depth show that predator maximum gape size, individual trophic position, and crucian carp size were the best variables explaining variation among predation categories (Tables 4 and 5, Figure 5). It is to be noticed that maximum gape size was also negatively correlated with crucian carp density. Littoral reliance, total phosphorus, predator proportion, and lake depth were excluded from the final model during model selection.

4 | DISCUSSION

The body shape of crucian carp differed significantly among the lakes investigated, and this variation was given mostly by differences in relative body depth. This difference in body shape was related to a gradient of predation risk represented by the predator community of each lake, which caused progressively deeper bodies, larger size, and lower population densities. Variation in body depth was related mainly to the maximum gape size reached by the predators in the different communities and crucian carp trophic position.

In general, in absence of predators, fish were smaller and had a more slender body shape and gradually showed higher absolute and relative body depth values when trout and perch were present, reaching the largest size and deepest bodies in pike lakes. Previous field studies, in line with our findings, show that crucian carp had a deeper body depth in populations sympatric with predators compared to allopatric ones (Poléo et al., 1995), but did not test the effect of specific predator communities. Experimental studies observed the effect of single predator species under controlled conditions and showed that crucian carp increased in body depth when exposed to cues from perch or pike and that the latter induced a more pronounced development (Brönmark & Pettersson, 1994).

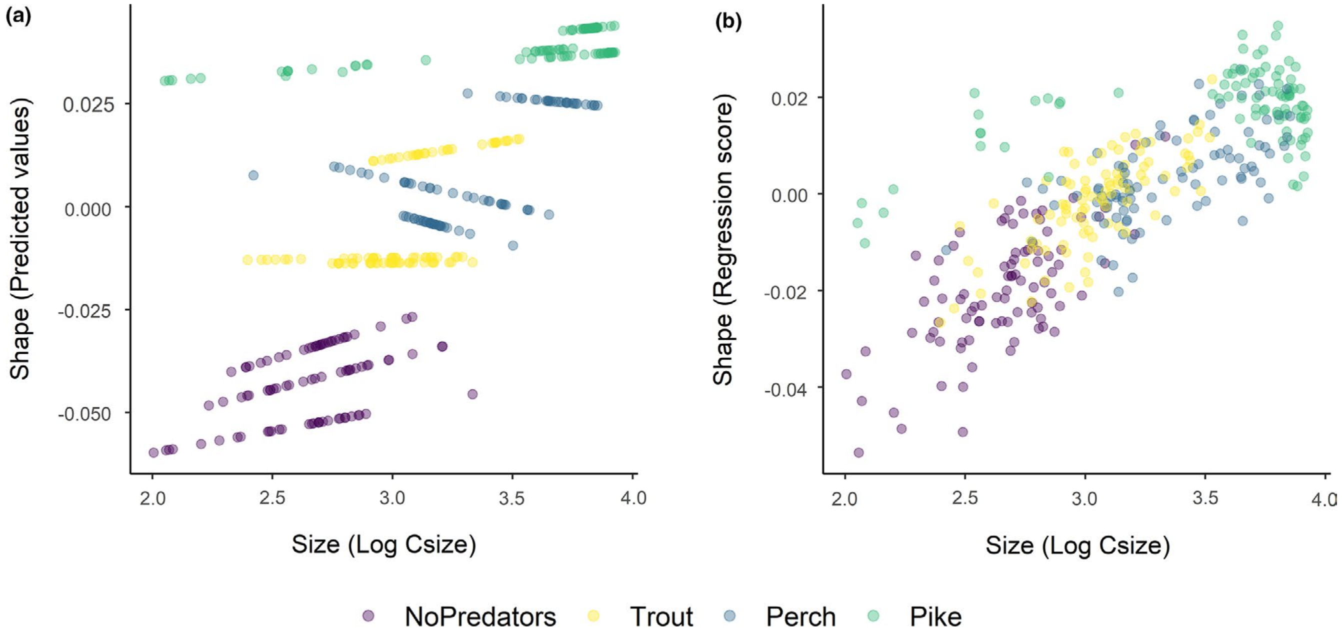

FIGURE 4 Allometric trajectories of crucian carp from twelve lakes with different predation regimes calculated using the *plotAllometry* function in the R package "Geomorph" (Adams et al., 2020). The x-axis values represent the log-transformed centroid size (LogCsize) as a proxy for individual body size. The y-axis values represent a) the shape as the first principal component of the predicted values and b) the standardized shape scores from the multivariate regression of shape on size

TABLE 4 Model selection for body height of crucian carp with biotic and abiotic environmental parameters as explanatory variables: predator proportion (RelPred), maximum gape size (MaxGS), trophic position (TP), littoral reliance (LIT), total phosphorus (TotP), maximum depth (MaxD), and body size (logCsize)

Model	AIC	ΔAIC	Wi
PC1 ~ MaxGS + TP + logCsize	−2,324.98	0.00	0.982
PC1 ~ MaxGS + TP + TotP + logCsize	−2,316.90	8.08	0.017
PC1 ~ RelPred + MaxGS + TP + TotP + logCsize	−2,308.73	16.25	0.000
PC1 ~ RelPred + MaxGS + TP + TotP + MaxD + logCsize	2,298.44	26.54	0.000
PC1 ~ RelPred + MaxGS + TP + LIT + TotP + MaxD + logCsize	−2,283.78	41.20	0.000

Note: AIC, difference in AIC (ΔAIC) and Akaike weights (Wi) for candidate models are shown.

TABLE 5 Results of the best linear mixed model (*PC1 ~ MaxGS + TP + logCsize*) explaining the relation between crucian carp body shape and maximum gape size (MaxGS), trophic position (TP), body size (logCsize)

Effect	Estimate	*SE*	*df*	*t*-*value*	*p*-value
(Intercept)	0.00002	0.0036	2.10	0.01	0.996
MaxGS	0.02700	0.0036	2.59	7.43	0.008*
TP	−0.00284	0.0008	354.00	−3.46	0.001*
logCsize	0.00289	0.0009	353.30	3.15	0.002*
Marginal R^2: 0.81; *Conditional* R^2: 0.94					

**p*-value < 0.05.

In our study, we also observed a smaller but significant increase in body depth of crucian carp from lakes with trout as the only predator present. Indeed, laboratory experiments showed that crucian carp was able to detect detailed information from waterborne cues such as predator diet or relative size. Individuals, for instance, showed different fright responses, as a decrease in swimming activity, when exposed to cues from large or small predators, or when these were fed crucian carp or invertebrates (Pettersson et al., 2000) Moreover, crucian carp exhibited different behavioral and neural responses to skin extract from trout, perch, and pike (Lastein et al., 2012). Fish also reduced activity levels and shifted to a nocturnal activity pattern when occurring with diurnal predators such as pike (Vinterstare, Hulthén, Nilsson, Nilsson, et al., 2020). These findings suggest that crucian carp may develop specific responses in presence of certain piscivorous fish species. A similar example of flexible predator-induced morphological defenses is represented by *Rana pirica* tadpoles, which develop a specific body shape in response to predators with different predation strategies (Kishida & Nishimura, 2005).

However, trout, perch, and pike lakes reflected a gradient in predation efficiency which was mainly defined by maximum mouth opening, making it difficult to distinguish the effect of predator community from the gape size. Still, perch in Lake Øvresetertjern and trout in the oligotrophic Lake Posttjernet reached the largest body and gape size relative to the other lakes with the same predator species. In these lakes, crucian carp had the highest body depth in relation to the other lakes from the respective predation category. Moreover, in Lakes Øvresetertjern, Svartkulp, and Posttjernet, where perch and trout made up more than half of the species present, crucian carp had a deeper body compared to the Lakes Bjørnmyrdammen, Småvanna, and Karussputten, where predators represented a smaller proportion of the total fish (14%–18%). However, in Lakes Stomperudtjernet and Nusttjennet, predators made up only a very small proportion of the total fish community (5%) and consisted mainly of few large pike and perch. Nevertheless, in these lakes, crucian carp reached the largest size and deepest body. In the presence of perch or trout, which undergo ontogenetic diet shifts to piscivory, crucian carp may grow considerably in body depth (Brönmark & Pettersson, 1994). On the other hand, pike, a largely piscivorous and highly efficient predator which share the same vegetated habitat with crucian carp, represents a constant threat. Thus, in presence of pike, crucian carp might have developed an effective adaptive response to predation risk, independently from its density. Moreover, in these lakes, predation risk might be intensified due to the presence of perch. The coexistence of perch and pike may impose a greater risk for crucian carp of different size classes both due to the greater gape size range but also to their very different foraging behavior (Eklöv & Diehl, 1994). Thus, our results support previous experimental studies suggesting that the development of a deep body represents a morphological defense against gape-limited piscivores (Nilsson & Brönmark, 2000). In particular, body depth determines prey size refuge, decreasing substantially vulnerability to predation (Nilsson et al., 1995). Moreover, this development in body depth would stop as soon as crucian carp reach the most functional morphology, that is, the size in which it is outside of the predation window. Indeed, in an experimental setting, removal of cues from predators resulted in a decrease in crucian carp relative body depth (Brönmark & Pettersson, 1994). While reaching a certain body shape in natural conditions is not directly comparable to the removal of predator cues in the laboratory, it suggests that a high body depth might be costly to maintain and that this development would be supported only when the predation risk is certain (Pigliucci, 2005). Thus, this variation in body shape does not seem to be the result of the simple exposure to predators, but more likely it is finely tuned with the specific structure and ecology of the predator communities (Holopainen, Aho, et al., 1997; Johansson & Andersson, 2009; Pettersson & Brönmark, 1997).

Regulation of development of body shape in response to predation risk seems to be a complex process, and indirect effects such as food availability and behavioral responses can also affect fish body morphology and growth at a fine scale (Pettersson & Brönmark, 1997; Svanbäck et al., 2017). In this regard, it was proposed that predator-induced morphological defenses are a by-product of prey

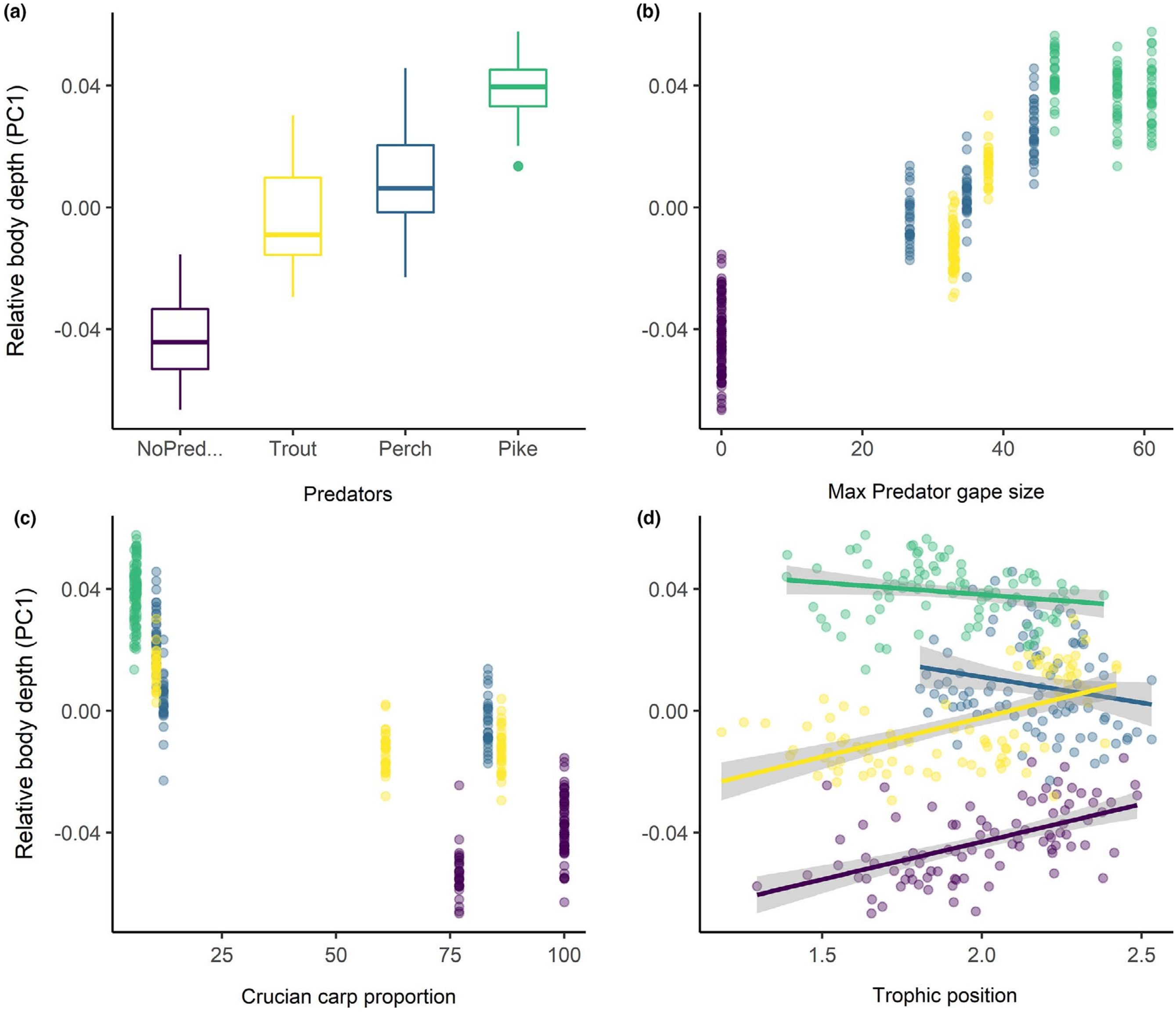

FIGURE 5 Relationship between crucian carp relative body depth (PC1) and (a) predation category, (b) maximum predator gape size (cm), (c) crucian carp proportion (%), and (d) trophic position

behavior, since predators can intimidate prey inducing a decrease in their foraging activity (Peacor, 2002). This reduction in movement has been hypothesized to lower prey metabolism with a reallocation of the energy saved to increased growth or development of defense structures (Bourdeau & Johansson, 2012). Other studies suggest a link between stress physiology and the expression of inducible defense traits (Middlemis Maher et al., 2013; Vinterstare et al., 2020). Our results show distinctly that progressively deeper bodies were accompanied by an overall increase in fish size. Moreover, crucian carp density was decreasing with increasing predation risk, which may have resulted in more available resources for surviving individuals. In presence of efficient predators such as pike, few large and high-bodied crucian carp were present. On the opposite, small-sized individuals occurred in higher densities in absence of predators. In this regard, piscivorous fish can affect the structure of prey communities and indirectly regulate resource availability through size-selective predation (Heynen et al., 2017). Predation can reduce prey density through direct consumption of small individuals, causing competitive release and eventually leading to an increase in somatic growth of survivors (Craig et al., 2006; Persson et al., 1996; Svanbäck & Persson, 2004). In contrast, dense populations in allopatric lakes have to compete for resource and their body condition remains low. This also suggests a potential higher growth rate with increasing predation risk (Vøllestad et al., 2004). Lake productivity also plays an important role in these dynamics, since it regulates resource availability and ultimately population density and somatic growth (Weber et al., 2010). Previous studies show that crucian carp achieved a deep body in a few months if low densities of shallow-bodied fish were introduced into a food-rich environment without piscivores (Holopainen, Aho, et al., 1997). However, discerning between the effects of predation and food availability is difficult in the present study, since the most productive lakes corresponded greatly to the ones with pike as main predator, making it difficult to isolate the two different effects. Remarkably, crucian carp from the allopatric pond Forkerudtjern, one of the most productive among the study systems, had the highest relative body depth respect to the other lakes with no predators, but fish were still considerably stunted, probably because of the high population density.

Individual resource use did not have a strong direct effect on crucian carp body shape. Trophic ecology of crucian carp was different among lakes, but our results do not show a clear shift in resource use induced by predation risk. Generally, fish seemed to rely on littoral invertebrates associated with substrate or vegetation, but at the same time, individual resource use varied greatly within each lake. A possible reason for the lack of correlation between body shape and resource use might be that many of the fish caught were already outside of the predation window and thus probably able to forage more actively and exploit different food resources. Moreover, fish might be able to easily use the resources from both the pelagic and littoral habitat since the study lakes were mostly small and both habitats are next to each other (Scharnweber et al., 2013). Furthermore, we did not catch any fish from the profundal habitat of deeper lakes—that is, maximum depth of around 11 m—suggesting that crucian carp were still confined to the shallow area. In contrast, crucian carp body depth was related to trophic position, and, in particular, different predator communities seemed to have specific effects. Trophic position had a positive influence on body depth in allopatric and trout lakes. With absent or low predation risk, one of the main limiting factors for crucian carp to feed on different resources could be mouth gape, as fish are able to exploit larger sized invertebrate resources only when they reach a certain body depth or size. In pike lakes, trophic position was slightly lower. Here, crucian carp hiding in the vegetation might feed on macrophytes and large invertebrates such as snails and clams, which likely lowers the trophic position when compared to zooplankton feeding. This also corresponds with a lowering of crucian carp activity, as fish expend less energy in foraging.

Crucian carp body depth increased along a gradient of predation risk represented by increasingly efficient predator categories. Specifically, our results indicate that crucian carp is provided with a fine-tuned morphological defense mechanism against gape-limited piscivores. The mechanism that triggers and regulates a change in body shape does not seem to be solely regulated by exposure to predators (Brönmark & Pettersson, 1994; Durajczyk & Stabell, 2014), but also depends on the specific structure and ecology of the predator communities. In many natural systems, prey organisms experience complex predation regimes. Species composition and abundance of predators can vary over time, especially in small lakes that are characterized by frequent fish mortality during winter (e.g., Lappalainen et al., 2016). In these small systems with high environmental stochasticity, plastic responses are advantageous since organisms are likely to be subject to strong interannual variability of predation pressure rather than constant predation risk (Kishida & Nishimura, 2006). Under such conditions, seasonal and annual changes in resource availability are also likely to occur, and generalist feeding strategies might be favored over specialization in acquiring specific resources (Scharnweber et al., 2013). Crucian carp flexibility in niche use is complex and needs to be better explored considering not only predation pressure, but also the competitive interactions and the abiotic conditions shaping these systems. In this sense, a limitation of this study was that the gradient of predation pressure corresponded to a shift in lake productivity and fish community, making it difficult to discern between the effects of predation risk and other environmental factors. For example, pike lakes were the most productive and had a complex fish community respect to the trout and perch lakes. However, this is an intrinsic characteristic of these systems, and crucian carp variation in body shape is likely a result of different ecological processes which act in synergy with specific predation risk. Moreover, though laboratory and field experiments show that this change in crucian carp body shape is mainly due to a plastic response, it could also be related to evolutionary responses, that is, natural populations may present differences in both their phenotypes and the extent of plasticity of those phenotypes as the product of natural selection within each population. Thus, further field and experimental studies should investigate if there is a genomic component to observed phenotypic differentiation.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

Ilaria de Meo: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (equal); supervision (lead); writing – original draft (lead). **Kjartan Østbye:** Conceptualization (equal); methodology (equal); resources (equal); supervision (equal); writing-review & editing (supporting). **Kimmo K. Kahilainen:** Conceptualization (equal); formal analysis (supporting); methodology (equal); supervision (equal); writing-review & editing (supporting). **Brian Hayden:** Conceptualization (supporting); formal analysis (supporting); resources (equal); supervision (supporting); writing-review & editing (supporting). **Christian H. H. Fagertun:** Conceptualization (supporting); investigation (equal); writing-review & editing (supporting). **Antonio B. S. Poleo:** Conceptualization (equal); project administration (lead); resources (lead); supervision (equal); writing-review & editing (supporting).

DATA AVAILABILITY STATEMENT

Landmark coordinates, stable isotope values, and environmental data used in this study are available on Dryad at https://doi.org/10.5061/dryad.d2547d825.

ORCID

Ilaria de Meo https://orcid.org/0000-0003-1433-2017

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The resource use of Crucian carp along a productivity gradient reveals dynamic adaptations contingent upon body size, predation risk, and competition

Ilaria de Meo[1], Kjartan Østbye[1], Kimmo K. Kahilainen[2], Brian Hayden[3], Marius Magnus[1], Antonio B. S. Poléo[1]

[1]Department of Forestry and Wildlife Management, Inland Norway University of Applied Sciences, Koppang, Norway

[2]Lammi Biological Station, University of Helsinki, Finland

[3]Biology Department, Canadian Rivers Institute, University of New Brunswick, Fredericton, NB, Canada

Correspondence: ilaria.demeo@inn.no

Abstract

Generalist fish species can feed on a wide resource spectrum and across trophic levels depending on changes in resource availability and intra- and interspecific interactions. Crucian carp (*Carassius carassius*) represents a good candidate species to investigate variation in the trophic ecology of generalist fish as it can be found in highly variable fish communities and its resource use is only relatively well documented. In this study, we explored the trophic ecology of crucian carp at the individual and population levels using stable isotope and gut contents analysis. We tested if resource use varied according to lake productivity, predation risk, intra- and interspecific competition, or individual fish size. We found that crucian carp resource preference was highly variable among and within lakes. In predator-free lakes, small crucian carp occurred in high densities, showed increased inter-individual specialization, and relied mainly on pelagic zooplankton. In presence of predators, large crucian carp occurred in low densities and included greater proportions of benthic macroinvertebrates in their diet. This shift in resource use was further favored in productive, shallow lakes where littoral prey were probably largely available. Interspecific competition was an important factor determining Crucian carp niche use, as fish had higher trophic position in absence of other cyprinids. Crucian carp showed highly dynamic resource use and food preferences in response to variable environmental conditions. Overlooking the complex diet preferences of generalist fish may lead to an oversimplification of freshwater community dynamics.

Keywords: Generalist fish, stable isotope trophic ecology, cyprinid, diet analysis, individual specialization

INTRODUCTION

Generalist fish species have often a fundamental role in aquatic ecosystems due to their ability to forage on a wide resource spectrum and across trophic levels, with profound effects on food web regulation and stability (Schindler & Scheuerell, 2002; Vander Zanden & Vadeboncoeur, 2002). Generalist feeding strategies are also beneficial in fluctuating environments since they allow fish to respond flexibly to changes in resource availability (Hayden et al., 2014; Pool et al., 2017; Laske et al., 2018). Accordingly, generalist fish are expected to vary their resource preferences along gradients associated with changes in environmental characteristics such as productivity (Lesser et al., 2020). In shallow temperate lakes, an initial increase in productivity intensifies the growth of benthic algae and macrophytes, enhancing invertebrate prey availability in the littoral habitat (Moss et al., 2004). As lakes become more eutrophic, nutrient supply often intensifies primary production of the pelagic habitat, with considerable changes in invertebrate and fish community structure (Jeppesen et al., 2000; Olin et al., 2002). Thus, the trophic ecology of species that can potentially behave as planktivores, benthivores, herbivores, or detritivores could change in response to resource availability in different systems (Araujo, Bolnick, & Layman, 2011; Vejříková et al., 2017).

At the same time, the ability of generalist fish to forage on alternative resources is strongly dependent on their intra- and interspecific interactions (Quevedo et al., 2009; Bolnick et al. 2010). Intraspecific competition can increase population niche variability as conspecifics may adopt different foraging behaviors and use distinct subsets of the population diet breadth if preferred prey items become less abundant (Svanbäck & Bolnick, 2007; Svanbäck et al., 2008). On the other hand, interspecific competition generally constraints niche width by limiting the range of available resources (Eloranta et al., 2013). The strength of interspecific competition will also depend on fish density and species composition present in a system, as closely related species are often assumed to have a similar ecological function and more likely to compete than distantly related ones (Burns & Strauss, 2011; Dayan & Simberloff, 2005). Predation is another important factor determining the trophic niche and population structure of prey (Brabrand & Faafeng, 1993; Brönmark et al., 2008). Predators can increase or decrease the strength of interspecific interactions, and thus resource partitioning, among competing prey

(Chase et al., 2002). Interspecific interactions also depend on abiotic characteristics such as habitat heterogeneity. Increased structural complexity of the environment, such as enhanced macrophyte density, usually leads to a decrease of predation pressure, as prey can adopt different antipredator behaviors and seek refuge in alternative habitats (Werner et al. 1983; Snickars et al., 2004). This change in habitat use is frequently accompanied by a diet shift, leading to decreased growth rates if less-favorable food resources are found (Werner et al., 1983). Moreover, competing species may occupy the same refuge or select sub-optimal habitats as the preferred one is already occupied (Mittelbach, 1988; Henseler et al., 2020). Foraging strategies can differ during ontogeny, as increasingly large fish have access to a broader range of resources (Scharf et al., 2000). Larger fish are also able to use different habitats, e.g., by attaining a size-refuge from predators, with a potential competitive advantage over smaller conspecifics (Byström et al., 2004; Woodward & Hildrew, 2002). Thus, body size can modify the strength of interspecific interactions in a way that competition or predation can be predominant at certain size classes (Persson, 1988).

Crucian carp (*Carassius carassius*) is a widespread generalist fish belonging to the Cyprinidae family and can inhabit a variety of habitats, ranging from small ponds to productive lakes with stagnant water. Due to a peculiar physiology, crucian carp is able to survive winter months in ice-covered anoxic water and is often the only fish species present in small lakes (Piironen & Holopainen, 1986). In these pond populations, high densities of small crucian carp lead to resource limitation and strong intraspecific competition (Holopainen et al., 1997; Pettersson & Brönmark, 1997). In contrast, in more complex fish communities, crucian carp occurs at low densities and individuals usually attain larger sizes. This fish displays an elusive behavior in lakes with piscivores (e.g. Vinterstare et al., 2020) and is well-known for developing a deep-bodied morphology in response to gape-size limited predators (Brönmark & Pettersson, 1994; de Meo et al., 2021). While the effect of predation risk on crucian carp resource use is relatively well-documented (Pettersson & Brönmark, 1993; Paszkowski et al., 1996; Andersson et al., 2006), the potential role of interspecific competition has often been overlooked. This species shows a wide diet spectrum, including zooplankton, benthic insect larvae, macrophytes, and detrital material (e.g. Penttinen and Holopainen, 1992). In warm, shallow, eutrophic lakes its diet can vary greatly and consists mainly of plant material and zooplankton or detritus, depending on the dominance state of the lake (Gao et al., 2017).

Benthic foraging of crucian carp can also cause resuspension of sediment and increase nutrient concentrations, with potential effects on lake trophic state and water quality (He et al., 2019).

Since crucian carp can be found along different productivity gradients, it represents a good candidate species to investigate the trophic ecology of generalist fish in small lakes. In this study, we consider how lake characteristics and fish community structure affect the resource use of crucian carp through stable isotope and gut contents analysis. Stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is a well-established method providing integrated information on consumer trophic ecology. Since isotope ratios of consumers and food sources are closely linked, variation in the environment that affects the isotopic values of food sources at the base of the food chain may also influence the position occupied by consumers in the niche space (Post, 2002; Newsome et al., 2007). Here, we explored the trophic ecology of crucian carp at the individual and population levels, taking into account the main environmental characteristics potentially underlying variation in isotope ratios of food sources among different lakes. In particular, we wanted to find out if resource use of crucian carp varied according to lake productivity, predation risk, intra- and interspecific competition, or individual fish size. We expected a shift towards more littoral resource use with increasing predator or competitor density, as vegetated littoral habitat represents both a refuge and an important food source. At the same time, we predicted this shift to be enhanced by increasing productivity and individual body size, as they influence availability and accessibility of littoral resources. We also expected an increase in individual specialization, and thus an increase in the population-specific niche size, in communities where crucian carp was the dominant species, and a narrowing in niche size with higher interspecific competition and predation risk. Thus, investigating resource competition, predation, and body size in lakes with different productivity and fish community structure, we aimed at revealing their relative contribution upon niche use of crucian carp.

METHODS

Study sites and data collection

The selected twelve study lakes and ponds were located in southeastern Norway and were relatively small (0.25-11 ha) and shallow (max depth 1.5-11.3 m; Table 1). Land use of the area surrounding the lakes was different among localities: peat bog-forest of birch or pine (lakes Posttjernet, Motjennet, Karussputten), forest with proximity to an urban area (lakes Øvresetertjern, Svartkulp, Småvanna, Langmyrtjern, Bjørnmyrdammen), and urban area or farmland (lakes Bugårdsdammen, Forkerudstjern, Stomperudtjern, Nusttjennet). Abiotic parameters included lake altitude (m a.s.l.), surface area (ha), maximum depth (m), littoral area (%), specific conductivity (S/cm), Secchi depth (m), total nitrogen (µg/l), phosphorus (µg/l) and organic carbon (mg/l). We estimated lake bathymetry in the field with a portable echosounder and the littoral area was calculated as the percentage of bottom depth shallower than 3.5 meters as a proxy for basin profile.

Fish collection was carried out between June and August in 2018 and 2019 during the open-water season to examine the food sources assimilated during the main period of fish growth and activity. We assessed fish density in each lake using Nordic multimesh gillnets consisting of 12 equidistant panels (mesh sizes 5–55 mm) for littoral and pelagic habitats. Moreover, different fishing methods were used to maximize the catch of small crucian carp (see details in de Meo et al., 2021). We calculated CPUE (*n* fish$\cdot$net$^{-1}\cdot$h^{-1}) for crucian carp (CPUE crucian), invertebrate-consumer fish potentially in competition for resources with crucian carp (CPUE comp), and piscivorous fish (CPUE pred; Table 2). Piscivorous fish species in these lakes were brown trout (*Salmo trutta*), European perch (*Perca fluviatilis*), and northern pike (*Esox lucius*). In particular, in lakes at these latitudes, trout and perch undergo ontogenetic shifts to piscivory at a size of around 20 cm (Jonsson et al., 1999; Jensen et al., 2012) and 17 cm (Hjelm et al., 2000, Horppila et al., 2000; Svanbäck & Eklöv, 2002), respectively. Consequently, large fish were considered predators. Permission to catch fish was given by the Norwegian Environmental Agency (2018/4155) and fish were sampled after oral approval by the local landowners.

We collected qualitative samples of zooplankton, benthic invertebrates, macrophytes, and sediment to estimate basal resources for stable isotope analysis (SIA). We sampled zooplankton hauling a plankton net through the water column in the pelagic zone of deep lakes or in the non-vegetated area of shallow lakes. Samples were later sieved through a 200-µm mesh. We collected benthic invertebrates from sediments and plants in the littoral habitat using kick nets and sorted them to the lowest feasible taxonomic level. Samples of sediment and of the most abundant macrophytes (mainly *Potamogeton* spp. and *Nuphar* spp.) were also collected from the littoral zone of each lake. Sediment comprised primarily decaying plant material.

In the laboratory, we measured individual fish total length (± 0.1 cm) and wet weight (± 0.1 g). A piece of dorsal muscle tissue was dissected from a subsample representative of the length distribution of crucian carp in each lake (*n*=30) and frozen at -20 ^{o}C for later stable isotope analysis (SIA). When available, we included smaller crucian carp to avoid underrepresentation of this size class.

Stable isotope analysis and rescaling

Fish muscle, invertebrate, plant, and sediment samples were freeze-dried at -50 ^{o}C for 48 h, ground to a homogeneous fine powder, weighed and encapsulated into tin cups. We analyzed stable carbon and nitrogen isotope ratios using a Costech 4010 elemental analyzer (Costech, California, USA) coupled to a Delta Plus continuous flow mass spectrometer (Thermo Finnigan, Bremen, Germany). Precision of internal standards was better than 0.1 ‰ for δ^{13}C and 0.2 ‰ for δ^{15}N. Elemental C:N ratios were low in all fish samples (3.3 ± 0.1) indicating low lipid concentrations and δ^{13}C values were arithmetically lipid-corrected prior to analysis (Kiljunen et al., 2006).

Since basal food isotope ratios varied greatly across lakes, it was necessary to take into account this variation when comparing changes in isotopic niche of crucian carp. In order to remove this variation not associated with trophic changes and make fish isotopic values directly comparable, we used a method based on modified Z-scores (Fry & Davis 2015). This method was adapted by Lejeune et al. (2021) to compare isotopic values of newts from different communities. Isotope values of consumers were standardized by rescaling raw isotope data into modified Z-scores using the mean and standard deviation of their prey community at each location. In this study, the mean of pelagic zooplankton, pooled benthic macroinvertebrates collected from the littoral area, and pooled plant and sediment were used as sources for calculation of the parameters (see Table S1 in Supplementary material). Rescaled stable isotope values are expressed using a Δ notation instead of δ notation and conserve the original measurement unit (‰ deviation from international standards).

Diet analysis

We examined the gut contents of all the fish used in stable isotope analysis. When feasible, we included more individuals to increase sample size, since fish with empty guts were excluded from the analysis. We estimated the proportion occupied by each prey category observed in the anterior third of crucian carp gut under a stereomicroscope using percentage scale. The observed prey items were grouped in three main categories: pelagic prey, benthic prey, and plant/detritus material. Pelagic prey included (I) pelagic cladocerans (mainly *Bosmina* sp., *Daphnia* sp.), (II) copepods (Calanoida and Cyclopoida), and (III) other rare pelagic prey items (chironomid pupae, water mites, Chaoborid larvae). Benthic prey comprised (IV) chironomid larvae, (V) gastropods, (VI) benthic cladocerans (mainly *Chydorus* sp.), and (VII) other benthic invertebrates (Odonata nymphs, Trichoptera, Ephemeroptera, and Coleoptera larvae, *Asellus aquaticus*, ostracods). We also included in a third category (VIII) plant (mainly filamentous algae and macrophytes) and (IX) sediment, largely composed of decaying organic material (Table 3).

Diet breadth was quantified using Levins' D index (Levins, 1968) standardized for resource richness. The index ranges between 0 and 1 and is used to show the relative level of diet specialization at the population level. Low index values characterize a population diet dominated by few prey items, while high values are indicative of a more generalist diet. In addition, we assessed individual dietary specialization (IS) calculating the mean overlap between the diet of each individual to that of its population (Bolnick et al., 2002). Values approaching 1 indicate low inter-individual diet variation, while values near 0 indicate high inter-individual specialization. We calculated Levins' D index and IS using the R package "RInSp" (Zaccarelli et al., 2013).

Statistical analysis

Environmental structure. We used the abiotic variables measured at each lake to explore potential environmental drivers of variation in the raw stable isotope values of basal sources

($\delta^{13}C$ and $\delta^{15}N$ of littoral invertebrates, zooplankton, plants, and sediments) and crucian carp among the different systems. Abiotic variables included lake altitude, surface area, maximum depth, littoral area, specific conductivity, Secchi depth, and nutrient concentration (Table 1). We identified main environmental gradients reducing the set of our abiotic variables using principle component analysis (PCA). Then, we explored association between the two first PC axes and basal resource values through correlation analysis (i.e. Pearson correlation coefficient).

Crucian carp resource use and niche width. In order to estimate the predominant resource use of crucian carp at the population level, we used Bayesian isotope mixing models from the R package 'simmr' (Parnell et al., 2010). Mean and standard deviation of pelagic zooplankton (PEL), pooled benthic invertebrates (LIT) and combined plant and detritus (SED) were used as baseline values. Trophic fractionation values of 3.4 ± 1.0‰ for $\delta^{15}N$ and 0.4 ± 1.3‰ for $\delta^{13}C$ were used in the model (Post, 2002). Difference in resource use of crucian carp among lakes was compared by looking at the overlap of the 95% credibility limits of each source. Moreover, we performed generalized linear mixed effects models to test for the effects of size (TL), productivity (PC1), and density of crucian carp (CPUEcrucian), competitor fish (CPUEcomp), and predators (CPUEpred) on individual $\Delta^{13}C$ and $\Delta^{15}N$ values, as calculated from the modified Z-scores. We also included interactions of crucian carp size with predator and competitor fish density and considered lake as random effect. The full model equation was: $\Delta^{13}C / \Delta^{15}N \sim PC1 + CPUEcrucian + TL*CPUEcomp + TL*CPUEpred$. Variance inflation factor (VIF) was used to detect potential multicollinearity. As values indicated low correlation ($VIF < 2.3$), all predictor variables were included in the full model. Selection of the best models was performed by backward elimination of fixed-effect terms based on the Akaike information criterion (AIC). Finally, we calculated the isotopic niche width of crucian carp in each lake as the standard ellipse area corrected for small sample sizes (SEAc) using 'Stable Isotope Bayesian Ellipses in R' (SIBER; Jackson et al., 2011). The isotopic niche space consisted of the individual $\Delta^{13}C$ and $\Delta^{15}N$ values. We interpreted differences in niche areas examining the overlap of the 95% credible intervals.

RESULTS

Environmental characteristics

Stable isotope values of basal sources (littoral invertebrates, zooplankton, plants and, sediments) and crucian carp varied greatly among the different lakes (Figure 1). At the population level, we observed the lowest $\delta^{15}N$ ratios for crucian carp in Karussputten (3.6 ± 0.4 ‰) and the highest in Stomperudtjernet (13.6 ± 1.2 ‰). The lowest $\delta^{13}C$ ratios were measured in Småvanna (-36.5 ± 1.4 ‰) and the highest in Øvresetertjern (-28.1 ± 0.5 ‰). The first two axes of the PCA accounted for 74% of the variation in the predictor variables (Figure S1). The first axis explained 56% of the variance and was loaded positively by all nutrient values and littoral area and negatively by maximum depth and transparency. Specifically, lakes were increasingly shallow, turbid, and productive along PC1 axis. Thus, we interpreted this axis as a gradient in lake productivity. The second axis explained 18 % of the variance and was positively loaded to specific conductivity and altitude and negatively to maximum depth and total organic carbon. We found positive correlations between productivity gradient and nitrogen values of fish and all baselines (Figure S2), as PC1 axis was positively correlated with $\delta^{15}N$ of crucian carp ($r=0.82$, $p<0.001$), zooplankton ($r=0.88$, $p<0.001$), littoral invertebrates ($r=0.72$, $p<0.001$), plants ($r=0.73$, $p<0.001$) and sediments ($r=0.54$, $p=0.006$). The correlation between productivity gradient and $\delta^{13}C$ was statistically significant only for crucian carp ($r=0.29$, $p<0.001$). We also found positive correlation between PC2 axis and sediment nitrogen values ($r=0.51$, $p=0.009$) and zooplankton carbon values ($r=0.39$, $p=0.024$). However, variation along this axis seems mainly driven by few relatively deeper lakes and correlations should be interpreted carefully.

Fish community

A total of 10 fish species was recorded from the study lakes. Crucian carp density and fish community composition showed different patterns (Table 2 and Figure S3). Small lakes were inhabited mainly by high densities of crucian carp (lakes 1 and 3), or by crucian carp and minnow (*Phoxinus phoxinus;* lake 2). Crucian carp coexisted with brown trout (lakes 4, 5 and, 8) or brown trout and perch (lakes 6 and 7) in mesotrophic lakes. Minnow also occurred in some of these lakes (lakes 2, 4, 7, and 8). In Bjørnmyrdammen (lake 9) only perch was present together with very low density of tench (*Tinca tinca*). In large and productive lakes (lakes 10, 11, and 12), perch and pike were the main predator fish species present. In particular, lakes 11 and 12 presented the richest number of species, including high densities of roach (*Rutilus rutilus*), bream (*Abramis brama*), and rudd (*Scardinius erythrophthalmus*) while perch and pike occurred at low densities.

Crucian carp resource use

In general, fish community structure, productivity and individual body size were all important determinants of crucian carp resource use. Individual body size was a significant predictor of both $\Delta^{13}C$ and $\Delta^{15}N$ values, while crucian carp density was excluded from model selection. Variation in $\Delta^{13}C$ values was mainly explained by productivity, predator density, and its interaction with crucian carp size (Table 4). In particular, $\Delta^{13}C$ values showed a strong positive association with productivity (Figure 2a). Small individuals showed great variation in $\Delta^{13}C$ values, while larger fish were more ^{13}C enriched (Figure 2b). In general, fish from lakes with high predator density were more carbon enriched, irrespective of their size. Results of model selection show that variation in $\Delta^{15}N$ values was explained mainly by fish size, competitor density, and their interaction (Table 4). Individuals below 15 cm in size were more ^{15}N depleted relative to larger crucian carp, irrespective of lake characteristics (Figure 2c). $\Delta^{15}N$ values of larger crucian carp varied greatly and seemed to depend on competitor fish density. At high competitor density, crucian carp had low $\Delta^{15}N$ values.

Bayesian mixing models revealed great variation in crucian carp resource use among populations (Figure 3). Reliance on pelagic and littoral resources did not show a clear pattern. In general, crucian carp from populations with small average body size seemed to rely more on pelagic rather than littoral invertebrates, and this separation was clear. Mottjennet represented an exception to this pattern, although gut contents revealed that pelagic prey, and particularly cladocerans, made up the largest proportion of the diet (Table 3). On the contrary, with increasing average body size, fish seemed to rely mostly on littoral invertebrates or on littoral and pelagic resources in similar proportions. Diet analysis confirms this pattern, as crucian carp included larger proportions of benthic prey with increasing body size (Figure 4). Reliance on plants and detritus increased in productive lakes (lakes 3, 11, and

12; Figure 3), which could contribute to explain the higher carbon values observed from generalized mixed models.

Crucian carp niche size

Standard ellipse area of $\Delta^{13}C$ and $\Delta^{15}N$ isotope ratios of crucian carp varied among lakes. Populations from predator-free lakes with small fish (lakes 1 - 3) and lakes with low productivity (lakes 4 - 7) had smaller isotopic niches compared to populations from more productive lakes (Figures 5 and S4). Fish from these lakes had also a very similar diet breadth index (Table 3). Bugårdsdammen had the largest isotopic niche area, although diet breadth and inter-individual variation appeared to be low. In this lake, however, benthic invertebrates made up 60.2% of the diet and were grouped in a single category even if they constituted a very heterogeneous group, including coleopterans and *Asellus aquaticus*, possibly underestimating diet breadth and inter-individual variation. On the opposite, Nusttjernet, a productive lake with large fish, presented a remarkably low isotopic niche area. Fish from this lake had also the lowest inter-individual variation in diet and very similar sizes. In particular, fish showed specialization on benthic cladocerans, mainly small bottom-dwelling chydorids, which accounted for more than 50% of the diet (Table 3). Chydorids were also the main diet item of crucian carp from Stomperudtjern, which had very similar environmental characteristics to Nusttjernet. Crucian carp from Stomperudtjernet, however, grouped clearly in large and small individuals in isotopic space, resulting in a wider niche area (Figure 5).

DISCUSSION

Crucian carp showed great variation in resource use. Productivity, individual body size, and interspecific interactions were all important underlying factors of niche use. Low densities of large fish were found in shallow lakes in association with different sympatric fish species, including top predators such as pike. In general, in these macrophyte-dominated lakes, crucian carp used more littoral prey sources. In contrast, small crucian carp were typical in lakes where other species were absent or present at very low densities. Here, pelagic zooplankton constituted an important part of their diet.

Crucian carp and all basal resources had increasingly higher nitrogen stable isotope values ($\delta^{15}N$) along the productivity axis. Nutrient inputs from agriculture or sewage usually elevate $\delta^{15}N$ ratio at the base of the food chain (Kendall et al., 2007), and thus increase $\delta^{15}N$ ratio of all consumers (Harrington et al., 1998; Botrel et al., 2014). This large variation in isotopic signatures is common in many other eutrophic lakes (e.g. Vuorio et al., 2006). Carbon stable isotope values of crucian carp showed also a weak positive correlation with productivity. Yet, even after accounting for this variation in the baselines, productivity represented one of the main driver of fish resource use. Reliance on carbon enriched resources increased along the productivity gradient, and fish included higher proportions of detritus and plants in the most eutrophic lakes. Fish community structure was also shifting from dominance of trout to perch and finally to high densities of cyprinids along the productivity gradient, as previously observed on broader spatial scales (Hayden et al., 2017). In turn, fish species composition, and in particular progressively efficient gape-size limited predators, can determine an increase in crucian carp body height and size (de Meo et al., 2021). Thus, trying to tell apart the main drivers explaining crucian carp resource use can be hampered by the fact that productivity and community structure were highly interconnected, although explanatory variables were not directly correlated in our study. Still, crucian carp naturally inhabits heterogeneous environments, so that in order to understand variation in the observed dietary patterns, it is necessary to consider these biotic interactions within their abiotic framework, without drawing mechanistic conclusions.

Body size was a determining variable in crucian carp resource use, as fish included more littoral prey in their diet with increasing size. In lakes where no potential predators were present, crucian carp occurred in high densities and small sizes. In these systems, fish relied mainly on pelagic resources. Diet analysis confirmed that pelagic zooplankton (mainly *Bosmina* sp.) represented an important food item in all lakes, while chironomids were always included in the diet in lower proportions. Crucian carp may undergo changes in diet during ontogeny associated with size-specific differences in their ability to ingest prey. Previous studies show that in allopatric lakes the preference for larger prey items increased with fish size, as large insect larvae (e.g. Odonata) were consumed exclusively by fish larger than 13 cm (Penttinen & Holopainen, 1992). Size-related shifts in feeding efficiency of crucian carp have also been demonstrated in the laboratory (Paszkowski et al., 1989). Our results confirm this pattern as almost all fish from predator-free lakes were below 13 cm and did not include large invertebrates in their diet. However, fish from these lakes also relied on other low-quality resources such as plants and detritus. In particular, in the eutrophic lake Forkerudtjennet, fish included a large proportion of filamentous algae in their diet, potentially contributing to the enrichment of individual carbon values. Consumption of few prey items such as filter feeding-zooplankton, detritus, or plant material and exclusion of large predatory benthic copepods or macroinvertebrates from the diet, could also explain the low trophic position and small niche size of crucian carp populations from these lakes. By contrast, larger crucian carp seemed not to be limited by gape size, as fish were including higher proportions of large macroinvertebrates in their diet with increasing body length.

Moreover, higher reliance on zooplankton could be associated with an increase in use of the open water zone of the lakes in absence of predation risk, whereas fish may be confined to the littoral area when a predator was present (Pettersson & Brönmark, 1993; Diehl & Eklöv, 1995). Nevertheless, fish included variable proportions of pelagic resources in their diet even in presence of predators, probably because large fish outside of the predation window would be able to move more freely between open water and shoreline and feed on resources from both lake areas (Werner & Hall, 1988).

Interspecific competition played an important role in crucian carp resource use. In lakes Nusttjernet and Stomperudtjernet, where high densities of different cyprinid species were present, crucian carp showed low individual specialization and included large proportions of small benthic cladocerans in their diet. Previous studies show that omnivorous common carp (*Cyprinus carpio*) and roach are competitor species, where the latter has a higher competitive edge on zooplanktivory (Nahon et al., 2020; García-Berthou, 2001). On the opposite, bream is a more efficient benthivore than crucian carp and is able to penetrate deep into the sediment and feed on benthic macroinvertebrates at large sizes (Lammens 1986; Persson & Hansson, 1999). Thus, crucian carp may have been limited to feed on a restricted subset of the total prey spectrum, as a result of resource partitioning with other cyprinids. Large proportions of small chydorids in the diet of crucian carp may also explain the low trophic position of fish from these lakes. Competition with minnow might also have influenced crucian carp resource use as they occupy mainly the shallower parts of the littoral zone and can maintain very high population density (Museth et al., 2002). Indeed, in Langmyrtjern, crucian carp occurred only with minnow and had the lowest average body size and highest specialization among all the study lakes, since fish were feeding either on detritus or on zooplankton. This suggests that the combined effect of high intra- and interspecific competition might have constrained crucian carp diet breadth and favored individual specialization, with negative effects on individual growth. Moreover, these competitive interactions may be intensified in presence of trout or perch, as minnow increase their use of shallow waters in presence of piscivorous species (Eklöv et al., 1994; Museth et al., 2010). In lakes Bjørnmyrdammen, Øvresetetjern, and Bugårdsdammen, where no other cyprinid species were present and small perch represented the main competitor species, crucian carp had distinctly higher nitrogen ratio and included larger proportions of macroinvertebrates in their diet, indicating utilization of different resources compared to the other lakes.

Moreover, as expected, in absence or low density of predators, intraspecific competition for food determined an increase of among-individual diet variation. By contrast, in productive lakes with predators, crucian carp occurred in low densities and resources were likely very abundant, resulting in low intraspecific competition and individual specialization (Svanbäck & Bolnick, 2007). At the same time, predation risk may have decreased the degree of individual diet variation as a consequence of restricted habitat availability and limitation of alternative food sources (Eklöv & Svanbäck, 2006).

Interestingly, crucian carp managed to reach very large body sizes even in lakes with high interspecific competition. This suggests that filter-feeding on small benthic zooplankton may represent an advantageous strategy for growth (Persson & Brönmark, 2002), even if previous studies show that the feeding apparatus of large carp species may be unsuitable to effectively retain small prey (Sibbing, 1988). Moreover, benthic cladocerans may be very abundant in these productive lakes and thus represent an easily accessible prey. However, the lack of data on resource abundance prevents us from estimating habitat profitability. Diet analysis should also be interpreted carefully since sampling was carried out at different times in the lakes over the summer.

Crucian carp changed resource use and food preferences according to variations in specific environmental variables. Fish included more littoral prey sources in their diet with increasing body size, fish community complexity, and lake productivity, all highly interconnected factors.

Small fish from allopatric populations, able to use the pelagic habitat in absence of predators, were relying predominantly on zooplankton. Big fish, probably released from gape-size limitation, were able to get larger proportions of benthic macroinvertebrates in their diet. Concurrently, predation risk likely determined an increase in littoral habitat use. Interspecific competition, in particular with other cyprinid species, was also an important factor determining resource preferences of crucian carp and should be considered in future studies in addition to the more recurrent predation risk. Resource partitioning with other cyprinids, however, did not seem to prevent crucian carp from growing to larger sizes. Previous studies revealed that variation in foraging preferences of cyprinids may have considerable implications for the ecology of freshwater communities, and caution against generalizing results from one size class to others (Driver, 2005; Nieoczym & Kloskowski, 2014), or from one system to others, since resource use seems highly context-dependent (Persson & Brönmark, 2002). Crucian carp showed high plasticity in resource use that reflected specific changes in community structure and productivity, suggesting that overlooking the complex diet preferences of generalist fish may lead to an oversimplification of the dynamics of these systems.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

IdM: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead). **KØ:** Conceptualization (equal); methodology (equal); resources (equal); writing-review & editing (supporting). **KKK:** Conceptualization (equal); formal analysis (supporting); methodology (equal); writing-review

& editing (supporting). **BH:** Conceptualization (supporting); formal analysis (supporting); resources (equal); writing-review & editing (supporting). **MM:** Conceptualization (supporting); investigation (equal); writing-review & editing (supporting). **ABSP:** Conceptualization (equal); project administration (lead); resources (lead); writing- review & editing (supporting).

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FIGURE LEGEND

Figure 1. Stable isotope biplots of δ^{15}N and δ^{13}C of crucian carp and basal food sources (means ± SD) from twelve lakes ordered by increasing productivity.

Figure 2. Relationship between crucian carp Δ^{13}C and (a) productivity (PC1) and (b) individual body size (cm), and between (c) crucian carp Δ^{15}N and individual body size (cm). The 95% confidence level interval for predictions from a linear model is shown for each plot.

Figure 3. Littoral (LIT), pelagic (PEL), and pooled plant and sediment (SED) resource use of crucian carp for each lake according to the Bayesian isotope mixing models. The box is drawn around the 25th and 75th quartiles, representing 50% credible intervals, while whiskers represent 95% credible intervals. Lakes are divided into communities without (1-3) and with predators (4-12) and arranged from left to right by increasing productivity within these categories.

Figure 4. Relative proportion of littoral prey, pelagic prey, and pooled plant and sediment in gut contents of crucian carp. Lakes are divided into communities without (1-3) and with predators (4-12) and arranged from left to right by increasing productivity within these categories.

Figure 5. Standard ellipse areas (SEA) representing the core isotopic niche space (p=0.95%) of crucian carp as determined through SIBER models. Lakes are divided into communities without (1-3) and with predators (4-12) and arranged from left to right by increasing productivity within these categories.

FIGURES

Figure 1

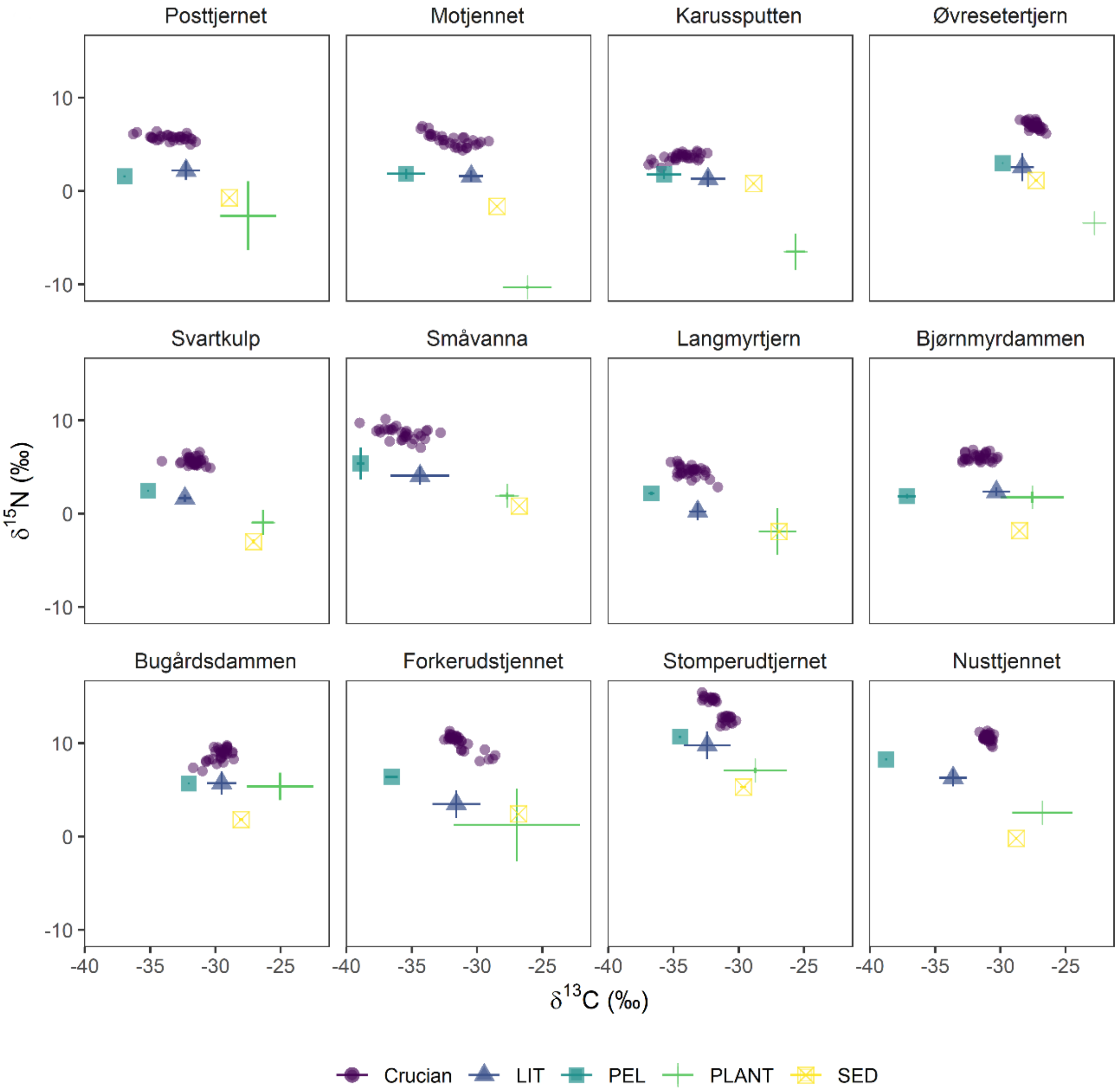


Figure 2

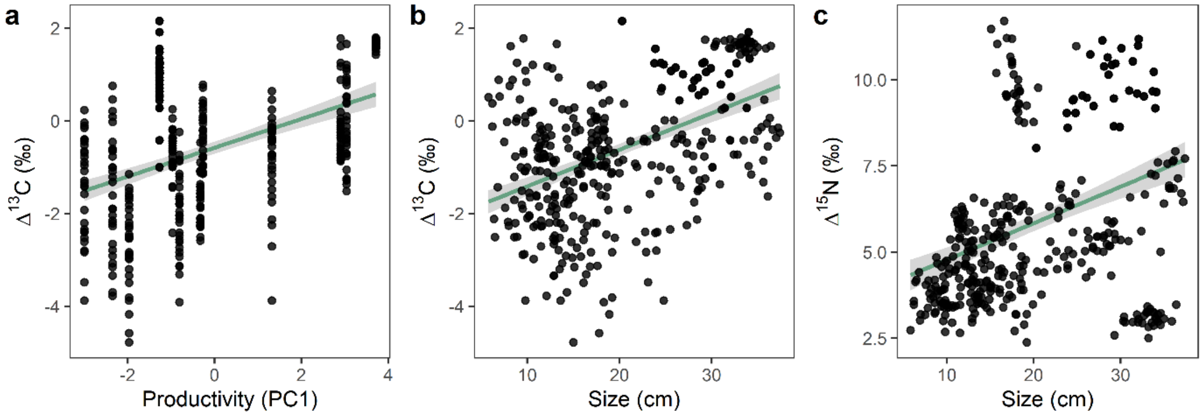
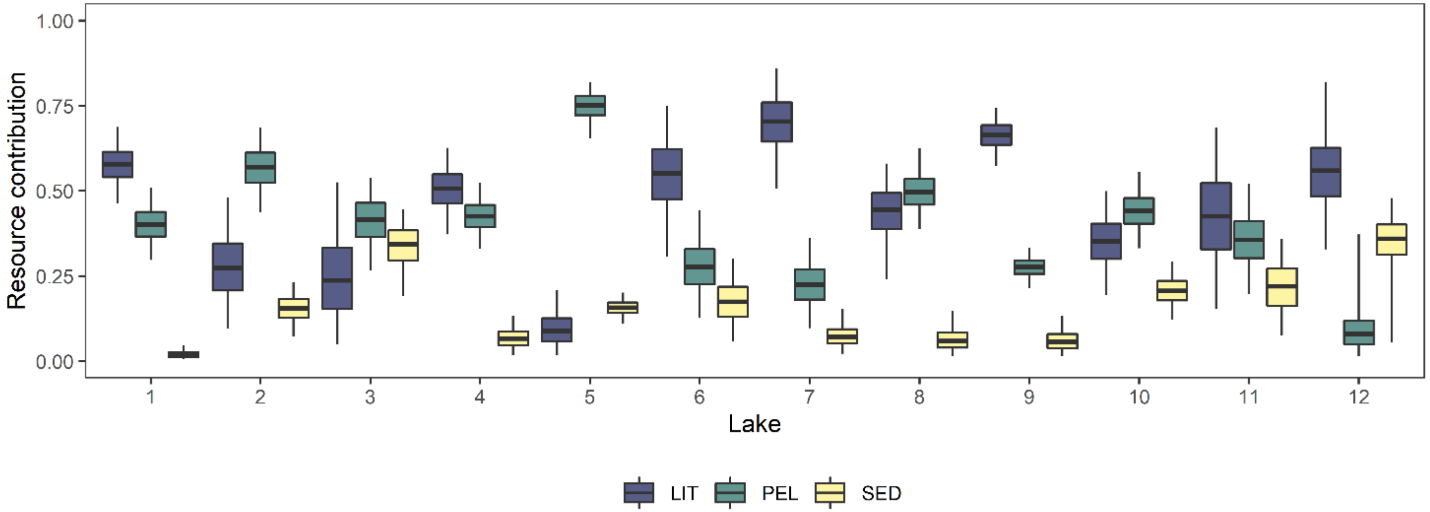

a
b
c
Δ^{13}C (‰)
2
0
-2
-4
-2
0
2
4
Productivity (PC1)
Δ^{13}C (‰)
2
0
-2
-4
10
20
30
Size (cm)
Δ^{15}N (‰)
10.0
7.5
5.0
2.5
10
20
30
Size (cm)

Figure 3



Resource contribution
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
8
9
10
11
12
Lake
LIT
PEL
SED

Figure 4

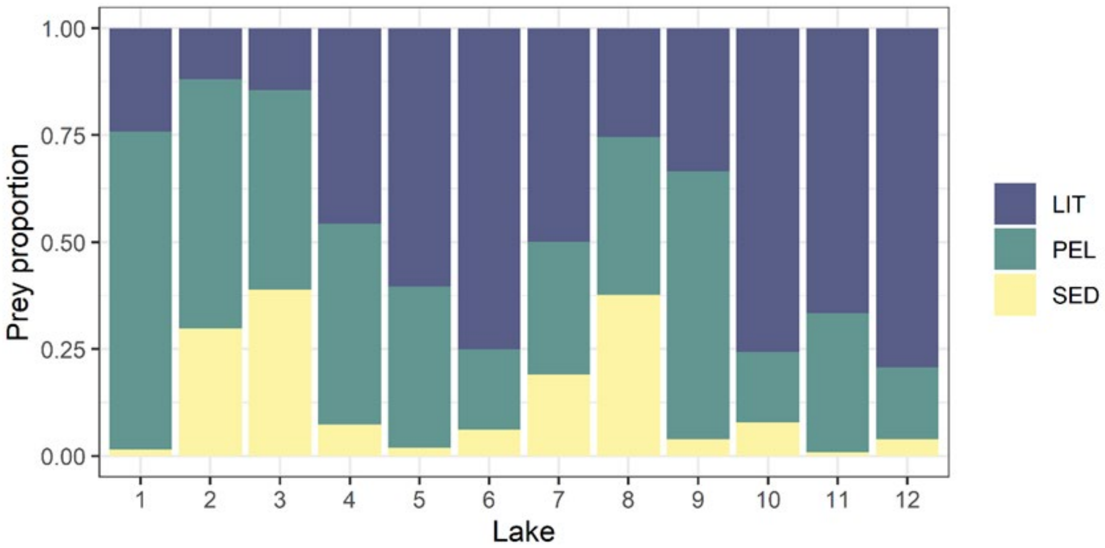
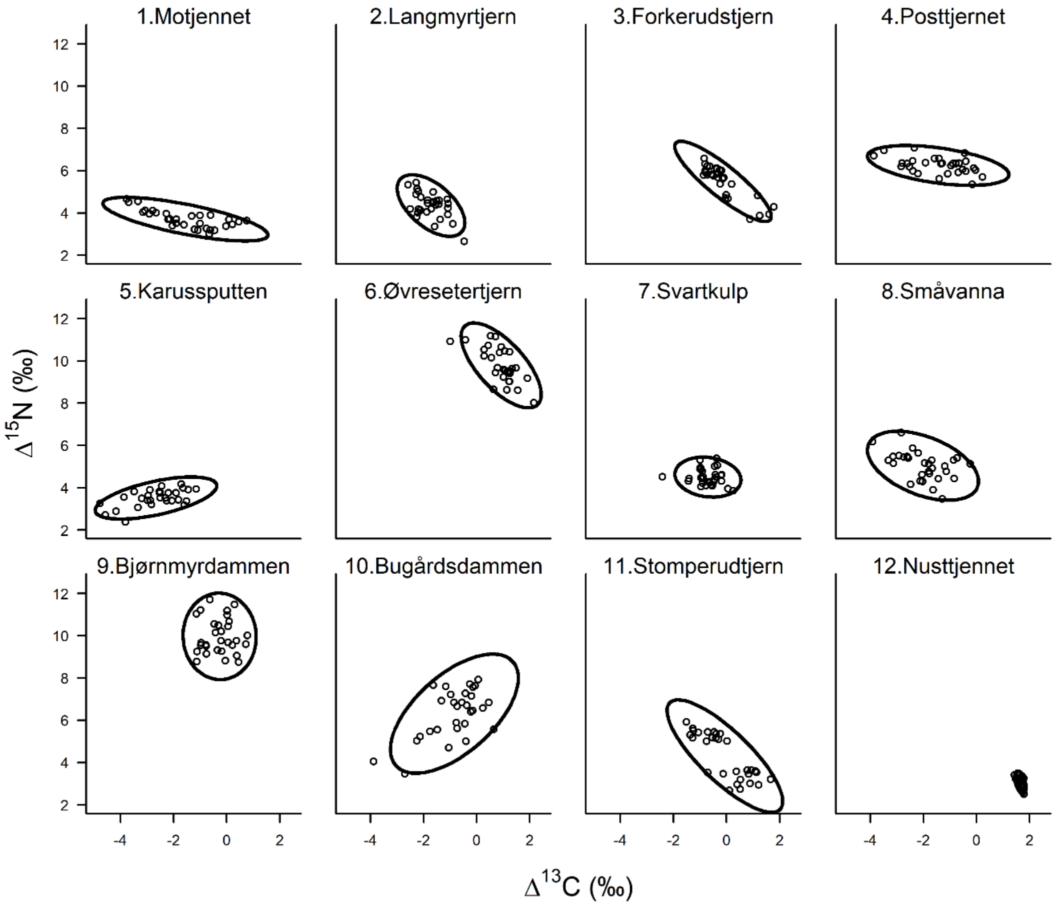

Prey proportion
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
8
9
10
11
12
Lake
LIT
PEL
SED

Figure 5


1.Motjennet
2.Langmyrtjern
3.Forkerudstjern
4.Posttjernet
5.Karussputten
6.Øvresetertjern
7.Svartkulp
8.Småvanna
9.Bjørnmyrdammen
10.Bugårdsdammen
11.Stomperudtjern
12.Nusttjennet
$\Delta^{15}N$ (‰)
12
10
8
6
4
2
-4
-2
0
2
$\Delta^{13}C$ (‰)

TABLES

Table 1. Environmental characteristics of the study lakes ordered by productivity gradient (PC1). Variables include latitude (Lat), longitude (Long), lake area (Area), maximum depth (MaxD), altitude (Alt), Secchi Depth (SD), littoral area (Lit), specific conductivity (SCond), total nitrogen (TotN), total phosphorus (TotP), total organic carbon (TOC). The first two axes of principal component analysis of environmental data are also included.

Lake	Lat (⁰N)	Long (⁰E)	Area (km^2)	MaxD (m)	Alt (m a.s.l.)	SD (m)	Lit (%)	SCond (S/cm)	TotN (µg/L)	TotP (µg/L)	TOC (mg/L)	PC1 55%	PC2 18%
Posttjernet	61.08	11.33	0.017	11	270.8	2.5	26	24.3	312	8	9.7	-3.00	-1.30
Motjennet	60.23	12.11	0.009	11.3	166.5	3.0	41	11.4	688	23	11.2	-2.35	-1.93
Karussputten	60.02	10.66	0.003	4.6	356.0	2.0	53	178.0	361	9	5.4	-1.97	2.01
Øvresetertjern	59.98	10.67	0.031	3.5	478.0	1.7	84	109.1	446	13	6.6	-1.27	1.84
Svartkulp	59.98	10.74	0.058	10	202.0	1.2	66	30.1	550	13	9.9	-0.97	-1.19
Småvanna	59.80	10.31	0.005	3.8	222.3	1.8	70	114.6	616	14	10.1	-0.81	0.80
Langmyrtjern	59.97	10.75	0.003	5	206.0	1.0	70	54.3	702	20	14.2	-0.33	-0.21
Bjørnmyrdammen	60.18	11.98	0.021	3.5	256.0	0.4	80	24.9	672	26	6.5	-0.27	0.34
Bugårdsdammen	59.13	10.19	0.050	2	42.0	1.5	100	129.4	980	54	9.5	1.32	0.47
Forkerudstjern	60.45	12.08	0.012	2.2	152.4	0.5	100	82.7	1985	82	23.4	2.90	-0.59
Stomperudtjern	59.32	11.40	0.038	1.5	103.4	0.4	100	186.0	1660	146	18.4	3.04	0.96
Nusttjennet	60.28	11.66	0.110	1.5	131.0	0.4	100	49.6	1090	164	16.4	3.71	-1.19

Table 2. Mean (±SD) total length (TL) of crucian carp, mean (±SD) catch per unit effort (CPUE) of crucian carp, predator, and competitor fish and number of fish species present in the study lakes.

Lake	**Lake n.**	**TL (cm)**	**Crucian CPUE (n·net^{-1}·h^{-1})**	**Predator CPUE (n·net^{-1}·h^{-1})**	**Competitor CPUE (n·net^{-1}·h^{-1})**	**n. fish species**
Motjennet	1	11.9 (3.0)	7.80 (5.08)	-	-	1
Langmyrtjern	2	10.7 (2.8)	2.07 (2.15)	-	0.62 (1.08)	2
Forkerudstjern	3	11.4 (1.9)	10.63 (6.5)	-	-	1
Posttjernet	4	19.3 (3.6)	0.19 (0.19)	0.01 (0.02)	0.76 (0.90)	3
Karussputten	5	14.5 (3.4)	0.99 (0.27)	0.01 (0.03)	0.14 (0.19)	2
Øvresetertjern	6	28.8 (3.6)	0.42 (0.61)	0.23 (0.30)	1.55 (2.21)	3
Svartkulp	7	19.8 (4.7)	0.30 (0.41)	0.16 (0.30)	0.64 (1.09)	4
Småvanna	8	15.4 (2.2)	1.03 (1.39)	0.03 (0.05)	0.32 (0.43)	3
Bjørnmyrdammen	9	18.0 (1.7)	2.11 (2.39)	0.02 (0.03)	0.20 (0.28)	3
Bugårdsdammen	10	31.5 (6.8)	0.10 (0.06)	0.17 (0.12)	1.23 (0.59)	3
Stomperudtjern	11	19.1 (9.5)	0.42 (0.34)	0.03 (0.03)	1.72 (2.51)	6
Nusttjennet	12	33.1 (1.6)	0.45 (0.26)	0.11 (0.12)	2.13 (3.19)	5

Table 3. Diet composition of crucian carp in the study lakes. Standardized Levin's index of trophic niche width, individual specialisation (WIC/TNW), number of full stomachs analysed (n), and Standard Ellipse Area (SEA) of crucian carp niche in isotopic space is also shown.

	Lake											
	1.Mot	**2.Lan**	**3.For**	**4.Pos**	**5.Kar**	**6.Øvr**	**7.Sva**	**8.Små**	**9.Bjø**	**10.Bug**	**11.Sto**	**12.Nus**
Prey items (%) (I) Pelagic cladocerans	58.1	57.8	40.6	44.3	30.6	14.5	15.8	33.6	60.4	12.8	30.1	13.4
(II) Copepods	16.1	0	5.1	0.1	0	3.6	2.1	3.2	0.6	0.3	2.5	3
(III) Pelagic invertebrates	0	0.4	0.9	1.7	7.1	0	13.3	0	1.5	2.4	0	0.1
(IV) Chironomid larvae	7.1	2.2	5.2	2.8	41.9	10.5	14.2	5.4	10.7	6.6	2.3	16.9
(V) Gastropods	0	0	0	1.7	0	3.6	0	0	0.4	5.9	0	1
(VI) Benthic cladocerans	6.5	1.7	0	8.2	8.5	6	6.2	5.3	9.3	4.5	58.8	50.8
(VII) Benthic invertebrates	10.5	8.1	9.2	34	10	55.9	29.4	14.8	13.3	60.2	5.5	10.9
(VIII) Plant	0	1.5	37.2	0	0.7	1.4	0	3.2	1.3	5	0.2	1.6
(IX) Sediment	1.6	28.3	1.7	7.2	1.2	4.5	19.1	34.5	2.6	2.4	0.7	2.3
Pelagic prey	74.2	58.1	46.6	46.1	37.7	18.2	31.2	36.8	62.4	15.5	32.5	16.6
Benthic prey	24.2	12.0	14.5	45.0	60.4	72.3	49.9	25.5	33.3	71.2	66.6	78.6
Sediment	1.6	29.8	38.9	7.2	1.9	5.9	19.0	37.7	3.9	7.4	0.9	3.9
Levins' D standardized	0.52	0.4	0.52	0.44	0.57	0.41	0.89	0.63	0.31	0.32	0.38	0.39
IS	0.53	0.22	0.54	0.78	0.52	0.76	0.72	0.54	0.55	0.81	0.68	0.9
n	41	32	53	22	42	25	30	33	27	25	53	45
SEA	1.18	0.80	0.94	1.14	0.97	1.19	0.59	1.58	1.43	2.82	1.79	0.05

Pelagic prey (%) was calculated as the sum of I, II and III, Benthic prey (%) as the sum of IV, V, VI and VII and Sediment (%) as the sum of VIII and IX.

Table 4. Results of the best linear mixed models explaining the relation between environmental variables and crucian carp $\Delta^{13}C$ and $\Delta^{15}N$ (‰).

Selected model: $\Delta^{13}C$ ~ Size * Predators CPUE + Productivity

	Estimate (±SE)	t-value	P-value
(Intercept)	-0.26 (±0.31)	-0.83	0.415
Size	-0.03 (±0.01)	-3.36	0.001*
Predators CPUE	0.15 (±0.32)	0.47	0.642
Productivity (PC1)	0.96 (±0.24)	3.91	0.003*
Size : Predators CPUE	0.03 (±0.01)	2.96	0.003*

Selected model: $\Delta^{15}N$ ~ Size * Competitor CPUE

	Estimate (±SE)	t-value	P-value
(Intercept)	4.01 (±0.65)	6.17	0.000*
Size	0.08 (±0.01)	8.84	0.000*
Competitor CPUE	-1.11 (±0.63)	-1.76	0.11
Size : Competitor CPUE	0.01 (±0.01)	2.45	0.015*

*p-value < 0.05

SUPPLEMENTARY MATERIAL

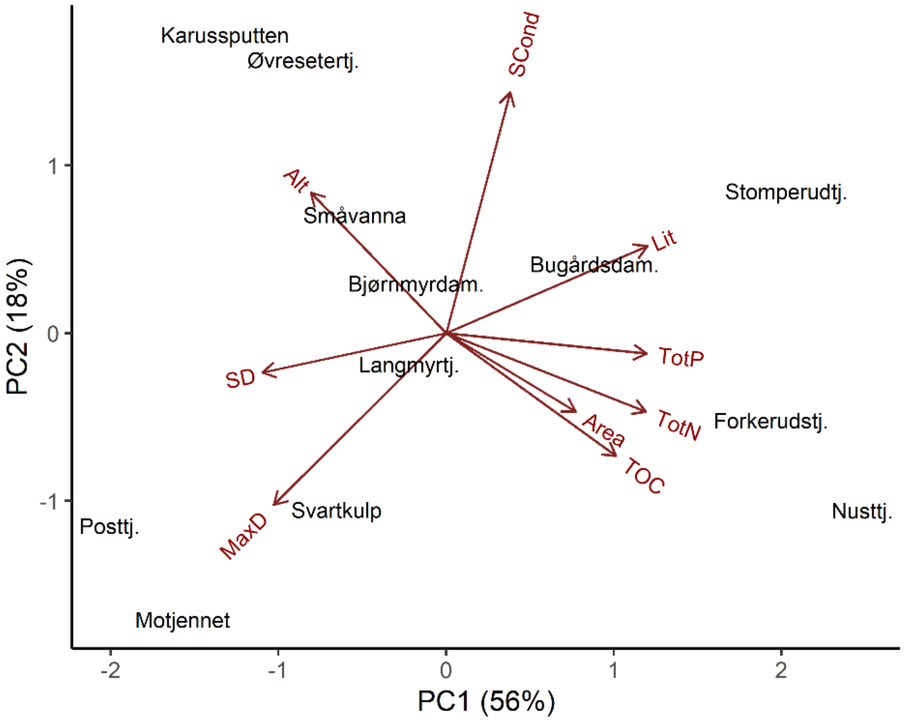


Figure S1. PCA bi-plot of the environmental variables lake area (Area), maximum depth (MaxD), altitude (Alt), Secchi Depth (SD), littoral area (Lit), specific conductivity (SCond), total nitrogen (TotN), total phosphorus (TotP), total organic carbon (TOC).

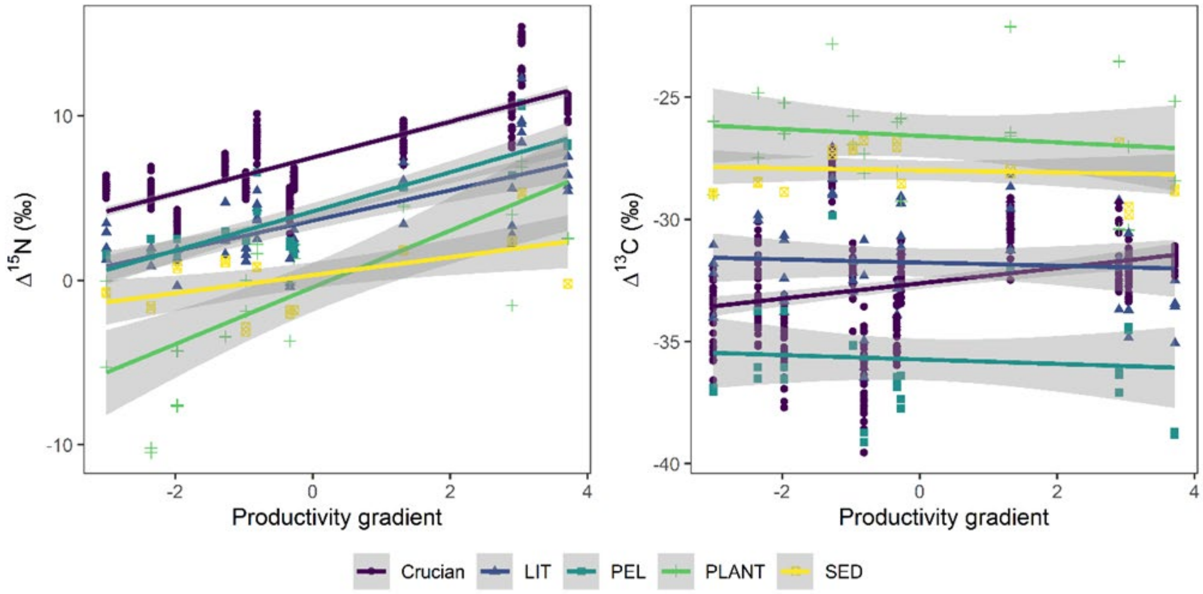


Figure S2. Scatterplots outlining the relationship of δ^{15}N and δ^{13}C of crucian carp and basal sources with productivity gradient (PC1), where nutrients increase towards more positive values. The shaded area represents the 95% confidence level intervals for predictions from linear models.

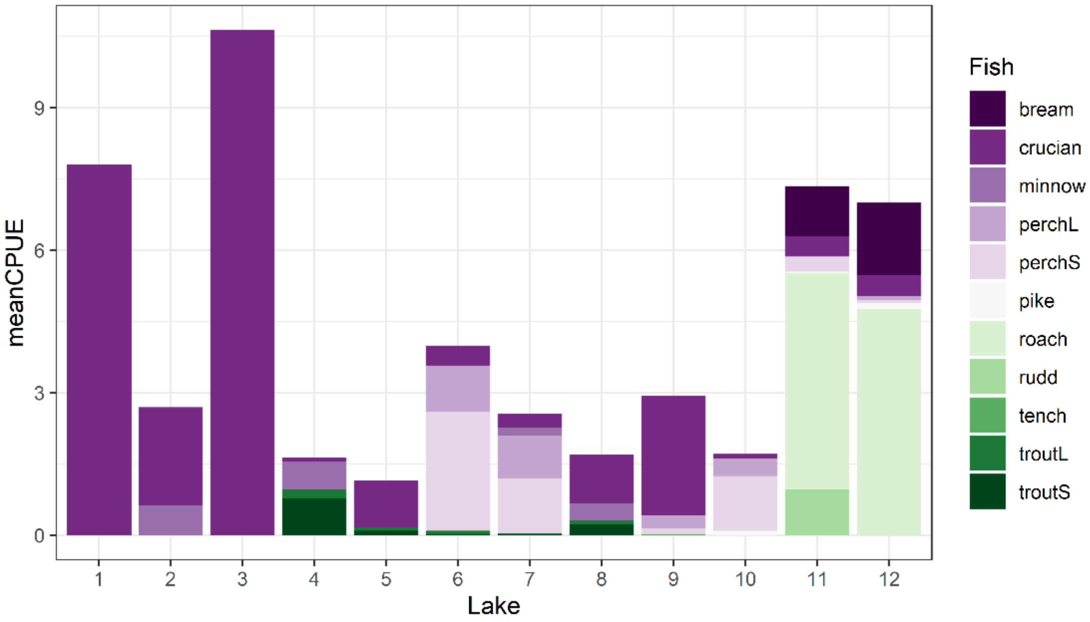


Figure S3. Mean density (CPUE) of each fish species in the study lakes. PerchL and troutL represent the density of large piscivorous perch (>17cm) and brown trout (>20cm), respectively.

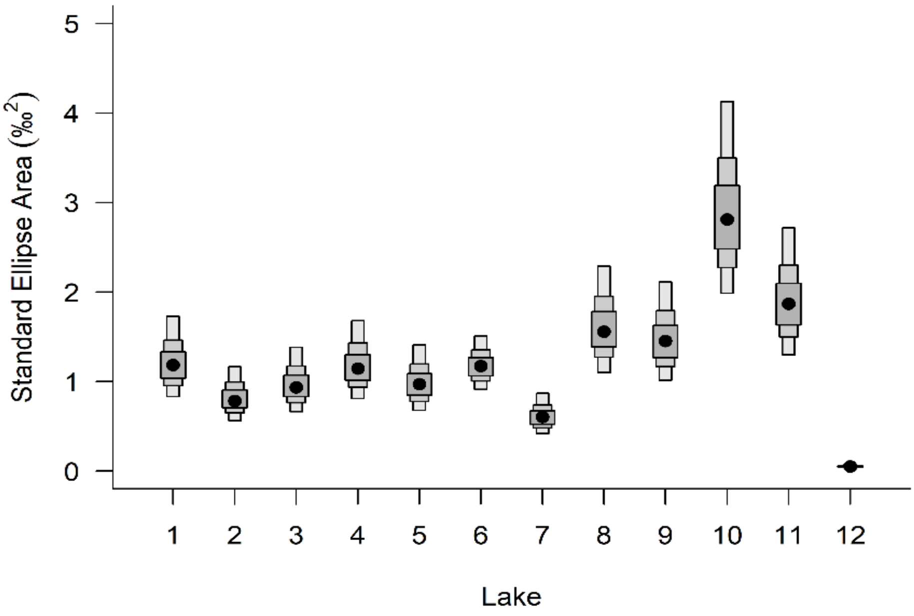


Figure S4. Estimates of Bayesian standard ellipse areas (SEAc) for crucian carp at each lake. Black dots correspond to the mean SEA for each group, and boxes represent the 50%, 75%, and 95% credible intervals Lakes are divided into communities without (1-3) and with predators (4-12) and arranged from left to right by increasing productivity within these categories.

3

Predation risk is the major driver of life-history changes of crucian carp in small boreal lakes

Ilaria de Meo[1], Kjartan Østbye[1], Kimmo K. Kahilainen[2], Antonio B. S. Poléo[1]

[1]Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Evenstad, Norway

[2]Lammi Biological Station, University of Helsinki, Finland

Correspondence: ilaria.demeo@inn.no

Abstract

Predation is a major evolutionary mechanism on life-history traits in prey organisms, but very little is known about trait responses with increasing predation risk in real-world settings. Here, we focus on the widely distributed species crucian carp (*Carassius carassius*). Crucian carp is known to be particularly vulnerable to predation and develops a deep body as an inducible morphological defense against gape-limited predators in experimental studies. We examined variation in growth and reproductive traits in 15 crucian carp populations along a predation risk gradient. We expected fish to attain higher growth rate, larger adult size, and later age at maturity with increasing predation. In absence of predators, we expected high adult mortality caused by strong intraspecific competition, which would lead to early sexual maturity and increased reproductive effort. We found that crucian carp life-history traits were clearly related to the presence of piscivores: fish grew older, attained larger asymptotic length and size at maturity with increasing predation risk. This size increment was evident at young age, especially in productive lakes with pike, and it suggests that fish quickly outgrew predator gape to reach a size refuge. Contrary to our predictions, populations had similar age at maturity, and reproductive effort was increasing with predation risk. Fish from predator lakes may experience high levels of resource availability as an indirect consequence of reduced intraspecific competition. High-predation lakes were also highly productive with high structural complexity due to macrophytes. These results suggest that profitable environmental conditions may favor a reduction in fish foraging activity and the allocation of energy to both growth and reproduction.

Keywords: Growth, reproductive effort, gape-limited predators, size refuge, predator-induced defenses, predation gradient

INTRODUCTION

In animals with indeterminate growth such as fish, there is a trade-off between somatic growth and reproductive allocation since fecundity increases with body size (Roff, 2002). Postponing maturity leads to higher fecundity in the future, but it is also associated to a potential decrease in fitness because of mortality prior to first reproduction (Stearns, 1992). In general, life-history theory predicts that fast growth and high mortality should lead to early maturity (Stearns, 1992; Haugen & Vøllestad, 2000). Moreover, once an organism reaches maturity, it has to allocate a certain amount of energy into reproduction that might not be available for growth or maintenance metabolism, with dramatic effects on future fecundity and survival (Bell, 1980). An individual with high reproductive effort early in life should have relatively short life span because the high investment in reproduction leaves no energy for surviving after spawning. By contrast, a species that invests little in reproduction should have higher life expectancy (Charnov, 1993).

Life-history strategies can vary greatly among fish populations of the same species and are strongly dependent on environmental factors such as latitude, length of the growing season, seasonal and interannual fluctuations, food availability, and interspecific interactions (Fox & Keast, 1991; Heibo et al., 2005; Blanck & Lamouroux, 2007; Walsh & Reznick, 2009). Predation has a central role in determining prey life-history traits, which will depend on the life stage at which predators prefer to select their prey (Belk & Hales 1993; Abrams & Rowe, 1996). In general, mortality on small individuals will select for late maturity and lower reproductive effort (Crowl & Covich, 1990; Reznick et al., 1990). Several mechanisms have been hypothesized to influence the direction of prey energy allocation in response to predation. In many species, prey growth or development of defensive structures (e.g. spines, plates) can be triggered directly by alarm cues associated with the presence of potential predators (Tollrian, 1995; Januszkiewicz & Robinson, 2007). These induced anti-predator defenses are considered a direct adaptive response to predation risk (Tollrian & Harvell, 1999; Beckerman et al., 2007). Predation can also determine an increase in individual growth rate of prey by reducing fish density, and thus, intraspecific competition (Tonn et al., 1992; Persson et al., 1996; Van Buskirk & Yurewicz, 1998). Predation risk can also indirectly induce shifts in prey behavior, such as a reduction in foraging activity or a switch to unprofitable habitats, resulting in lower growth or reproductive allocation (Lima et al., 1990; Heins et al., 2016). Alternatively, prey can increase foraging activity and undergo higher predation risk at small sizes in return for faster growth and low mortality as soon as they outgrow predator's handling abilities (Urban, 2007). Moreover, variation in life-history strategies within the same species has been related to personality and physiological traits (Biro et al., 2004; Réale et al., 2010). The choice of a certain behavior is also highly dependent on predation tactics (Van Buskirk, 2001; Wood & Moore, 2020) and resource availability since scarce food generally forces individuals to increase foraging activity or use of risky habitats (Werner & Hall 1988; Anholt & Werner, 1995).

A fast growing life-history strategy can be particularly advantageous in presence of gape-limited predators. Gape-limited predators usually select prey below a certain size that maximizes their capture and handling ability (Hambright, 1991; Nilsson & Bronmark, 2000). This size-selective mortality on small individuals may favor prey species growing rapidly into a ''size-refuge'' (Lundvall et al., 1999; Taborsky et al., 2003). Accordingly, prey reaching a size-refuge to elude gape-limited predators are subject to higher juvenile mortality, and rapid somatic growth may be achieved by postponing reproduction. This strategy was observed among populations of bluegills (*Lepomis macrochirus*), which grew faster and reached sexual maturity at a relatively larger size and older age when occurring with high abundances of large predators (Arendt & Wilson, 1999). Other studies showed that different populations of guppies changed their life-history patterns depending on the type of predation. In lakes with gape-limited predators, guppies matured at a later age and larger size and had lower reproductive effort when compared to guppies occurring with large predators feeding mostly on adult individuals (Reznick & Endler, 1982).

Crucian carp (*Carassius carassius*) is a widespread cyprinid fish particularly vulnerable to gape-limited predators such as perch (*Perca fluviatilis*) and pike (*Esox lucius*) (Brönmark & Pettersson, 1994; Vinterstare et al., 2020). Crucian carp develops a deep body when exposed to cues from predators feeding on conspecifics, representing an inducible morphological defense against predation risk (Nilsson & Brönmark, 2000). In natural populations, crucian carp has usually been described as occurring with a distinct dichotomy in morphology, biology, and population structure, depending on piscivore presence (Holopainen et al., 1997a). This fish has physiological adaptations to survive anoxic conditions under ice cover during winter. In autumn, it builds up a glycogen storage that can be used for anaerobic metabolism (Blažka, 1958; Piironen & Holopainen, 1986). Since most other fish species are sensitive to oxygen depletion, a lake may change from a multispecies assemblage to a monospecific assemblage of crucian carp under severe winter conditions. In lakes without predators, crucian carp can reach very high densities, and have low relative body depth and small size. By contrast, in lakes with piscivores, crucian carp have greater relative body depth and typically occur in low density and large sizes (Brönmark et al., 1995; Poléo et al., 1995). However, variability in crucian carp body form seems to range within these two morphotypes, as increasingly efficient predator communities induce progressively deeper body shape and larger size (de Meo et al., 2021). Experimental studies have shown that this change in body depth could be associated with resource availability and fish behavior rather than with predation cues only. High densities of crucian carp in predator-free lakes are subject to strong intraspecific competition, eventually leading to a stunting of populations (Tonn et al., 1994). Moreover, crucian carp respond to alarm cues by decreasing swimming activity, suggesting an alteration in energy use with allocation to somatic growth in presence of predators (Holopainen et al., 1997b; Pettersson & Hedenström, 2000). Whether these changes in growth and morphology are directly or indirectly mediated by predation, a deep body and large size seem to be the result of better growth conditions (Vøllestad et al., 2004). However, to our knowledge, there are no studies testing the effect of different predation risk levels on life-history traits of crucian carp in the wild.

In this study, we examined variation in life-history traits of crucian carp from 15 lakes along a gradient of predation risk represented by piscivore populations with increasing gape size. The different predator communities included brown trout (*Salmo trutta*), perch, and pike. Studies on growth and life-history traits of crucian carp populations in northern Europe are currently limited to predator-free populations (Tarkan et al., 2016 and references therein). With increasing predation risk on small individuals, we expected fish to attain higher growth rate and larger adult size. We supposed that this increase in growth may be achieved by delaying age and size at maturity. On the opposite, in absence of predators, we expected higher mortality of adult individuals because of strong intraspecific competition, associated with early age and size at maturity. We also examined how reproductive effort, in terms of relative gonad weight, varied with predation risk and we predicted that it would be higher in absence of predators because of shorter life span and opportunity for reproduction.

METHODS

Study lakes and data collection

The 15 study lakes were located in southeastern Norway and were surveyed between June and August in 2018 and 2019 (Table 1). All lakes were small (0.25 - 11 ha), had a high proportion of littoral zone, and variable productivity. Abiotic parameters included lake surface area (ha), maximum depth (m), total nitrogen (µg/l), total phosphorus (µg/l), and total organic carbon (mg/l). These were collected directly in the field, or retrieved from the Norwegian Environment Agency and the Norwegian Institute for Water Research.

Lakes presented different communities of piscivorous fish. Six lakes were completely predator free (Forkerudtjern, Mottjernet, Langmyrtjern, Nordre Sætertjern, Skråtjernet, Brynitjernet), whereas nine lakes had different gape-size limited predators: brown trout (Karussputten, Småvanna, Posttjernet), perch (Bjørnmyrdammen), perch and brown trout (Svartkulp and Øvresetetjern) or perch and pike (Nusttjernet, Bugårdsdammen, Stomperudtjern). Fish were caught with various sets of gillnets, baited traps, and kick nets to maximize the chance to capture small crucian carp. The relative fish density was assessed using Nordic multimesh gillnets 1.5 m deep and 30 m long, consisting of 12 equidistant panels with mesh sizes of 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm from knot to knot. We calculated CPUE (*n* fish·net^{-}1·h^{-1}) for littoral and pelagic habitats (for details see de Meo et al., 2021). After capture, fish were euthanized with an overdose of the anesthetic tricaine methanesulfonate (MS-222) by prolonged immersion, transported to the laboratory, and frozen for subsequent analysis. Permission to catch fish was given by the Norwegian Environmental Agency (2018/4155) and fish were sampled after oral approval by the local landowners.

Laboratory work

We measured crucian carp for total length (TL) and body depth (BD) to the nearest mm, and wet weight (W) to the nearest 0.1 g. Body depth was measured from the anterior insertion of the dorsal fin to the insertion of the pelvic fin. We determined sex and maturation stage of crucian carp by gonad inspection. We considered females mature when gonads were turgid and presented visible oocytes. We recorded wet weight of mature gonads to the nearest 0.001 g. For age determination, we took a sample of scales from the area between the dorsal fin and the lateral line. We also removed and cleaned both opercular bones for aging. Age was determined by counting true annuli along the primary radius of scales (*n* ~ 6) using a Microfiche Reader (Eyecom 1000). We excluded damaged or regenerated scales from the reading. For large individuals, we used both scales and opercula to increase aging reliability. In addition, we measured total length and gape height of brown trout, perch, and pike (Table 1). We considered gape height as the maximum distance between the tip of the upper jaw and the mandible. We calculated the mean maximum gape height for each lake by selecting the piscivores with the largest gape (*n* = 10). A large proportion of brown trout and perch in the study lakes was small-sized and probably did not undergo ontogenetic shifts to piscivory (Hjelm et al., 2000; Jensen et al., 2012). Thus, we selected maximum gape size over mean gape height to avoid potential underestimation of the maximum predation window.

Data analysis

Total length, relative body depth, and condition

We calculated individual crucian carp relative body depth (RBD) as the ratio of body height to total length. Fish body condition was assessed using Fulton's condition factor (K=body mass/total length^3 × 100). Differences in mean total length (TL) and relative body depth (RBD) among crucian carp from different lakes were tested with Kruskal-Wallis test. Fulton's condition factor was highly correlated with relative body depth (Pearson: r=0.91, p-value <0.001) and was excluded from further analysis. Differences in mean total length and relative body depth between males and females from each lake were tested with a Mann-Whitney test. Significant deviations from the expected male-to-female sex ratio (1:1) were tested using the chi-squared ($\chi 2$) test. When possible, we used only data from gillnets to avoid sampling bias in sex ratio, as females entering a trap may attract several males. All statistical analyses were conducted using R version 4.0.1 (R Core Team, 2020).

Growth

In this study, we estimated growth parameters using a Bayesian approach through the 'BayesGrowth' package (Smart & Grammer, 2021) in R statistical software. In general, fish sampling was biased towards large individuals, as small crucian carp show an elusive behavior, especially in lakes with predators. In this case, Bayesian models can be particularly useful since they can account for underrepresentation of the smallest size classes. The ‘BayesGrowth’ package uses the ‘Stan’ computer program (Carpenter et al., 2017) to build Markov-Chain Monte Carlo (MCMC) models via the ‘rstan’ R package (Stan Development Team, 2020). We fitted three common growth models to the data from each lake: the von Bertalanffy growth function (VBGF), the Logistic function, and the Gompertz function (see Table S1 in Supplementary material). We used informative priors on L_0 and L_∞ parameters with a normal distribution. L_0, which represents the length-at-birth, was set at 0.5 cm for all lakes (Laurila et al., 1987). L_∞, which represents the asymptotic length at which growth is zero, was set at 38.1 cm, the maximum individual length recorded from all lakes. Standard error on priors was set at 10%. We used the same prior for the three growth functions. We ran four MCMC chains with 10000 iterations and a burn-in period of 5000 iterations. Model selection was achieved through leave-one-out-cross-validation (LOOCV) method using the ‘loo’ R package (Vehtari et al., 2017). LOO-weights were calculated for each model, and the candidate model with the highest LOOICw was considered the most appropriate. In subsequent analysis, we considered only the parameters estimated from the best growth model. When enough data were available, we fitted two different growth curves for males and females for each lake. We checked for model convergence using the Gelman–Rubin test and with diagnostic plots using the ‘Bayesplot’ R package (Gabry, 2020).

Reproductive biology

The length (L_M) and age (A_M) at which 50% of individuals became sexually mature was determined by fitting a logistic model to the proportion of mature vs immature using generalized linear models. Confidence intervals for the model parameters of the logistic regression were estimated via bootstrapping (n=1000). L_M and A_M were determined for both sexes combined, males and females in predator-free lakes (Forkerudtjern, Mottjernet, Langmyrtjern, Skråtjernet and Brynitjernet) and in Karussputten. In the other lakes, most of the fish were mature and we were able to estimate length and age at maturity only for combined sexes for Stomperudtjern and Svartkulp. Moreover, we calculated gonadosomatic index (GSI) for females as (gonad mass/somatic mass*100), where somatic mass (g) is the total mass excluding gonad mass (Bagenal & Tesch, 1978).

Mortality

The instantaneous (Z) and total annual (A) mortality rates were estimated by catch-curve regression method. Z is the negative slope of a linear regression model fit to the natural logarithm of catch at age. We included only those age classes that were presumably fully recruited to the gear considering the descending limb of the catch-curve (Miranda & Bettoli, 2007). We used combined data from gillnets and traps, as there were no significant differences in mean total length of fish collected by the two gears (paired t-test; $t = -1.31$, p-value = 0.22). Lakes with small sample size or a narrow range of recruited ages were excluded from the analysis. We used the 'FSA' R package for analysis (Ogle et al., 2021).

Relating predation risk with life-history traits

We related predator maximum gape size to growth and reproductive parameters using Spearman's rank correlation. We used Bootstrapped estimates (n bootstraps = 1000) for the estimates of correlation coefficients. Spearman correlation analysis was selected over more complex approaches as sample sizes were low in some cases. Growth parameters included the growth coefficient (k), asymptotic body length (L_∞), length at age 1 (L_1) and age 3 (L_3), and maximum life span (T_{Max}). Length at age 1 and 3 were chosen as representative of initial growth effort to avoid predation. Maximum life span was calculated as the average maximum age observed in each population (n=10). Reproductive parameters comprised length (L_M) and age (A_M) at maturity, gonadosomatic index (GSI), and sex ratio. We also calculated the reproductive life span (RT) as the difference between the observed maximum life span and estimated age at maturity. Relative body depth (RBD) was also included in the analysis.

RESULTS

Total length and relative body depth

Crucian carp mean relative body depth differed significantly among lakes ($\chi^2 = 1473.7$, d.f. = 14, $p < 0.001$) and ranged from 0.27 in Langmyrtjern to 0.40 in Bugårdsdammen, Nusttjennet, and Stomperudtjernet, where both perch and pike were present (Table 2). Mean total length was also significantly different among lakes ($\chi^2 = 1467.7$, df = 14, $p < 0.001$). Maximum total length ranged from 11.7 cm in Politihøgskolen to 38.1 cm in Bugårdsdammen. In all lakes without predators, Karussputten and Småvanna, females were significantly larger than males, but relative body height did not differ between sexes (Figure 1). In the other lakes, females had similar mean total length or were slightly larger than males (Bjørnmyrdammen and Nusttjernet). By contrast, males had similar or higher relative body depth than females, with the exception of Øvresetetjern (Figure 1). Sex ratio was significantly different among lakes ($\chi^2 = 91.97$, df = 14, $p < 0.001$). Females were always dominant in predator-free lakes, with the exception of Mottjernet. In presence of predators, sex ratio was generally in favor of males and presented the highest values in lakes Bugårdsammen and Stomperudtjernet (Table 2).

Growth

For each lake, results of model selection by LOOIC showed that the von Bertalanffy growth function was the best-fit growth model for age estimation (LOOICw =1), while there was no support for the Logistic (LOOICw = 0) and the Gompertz growth functions (LOOICw = 0). Thus, all parameters used in the following analysis were estimated from the von Bertalanffy growth function. Mean parameter estimates varied greatly among the various lake (Table 2 and Figure 2). L_∞ ranged from 12.6 cm in N.Sætertjern to 42.0 cm in Bugårdsdammen. The growth coefficient was the highest in N.Sætertjern ($k = 0.32$ year^{-1}) and lowest in Brynitjernet ($k = 0.07$ year^{-1}). It has to be noted that in lake Brynitjernet fish seemed to reach very large asymptotic length compared to other predator-free lakes (L_∞=31.9 ± 4.1). However, large standard deviation suggests that adult fish might be undersampled in this lake. L_∞ and k were negatively correlated ($r = - 0.62$, $p < 0.013$). L_∞ values were generally higher for females, while males had higher growth coefficient (Table S2). Maximum life span ranged from 6.1 years in N.Sætertjern to 16.2 years in Bugårdsdammen.

Reproductive traits

We found no significant differences in length (paired t-test; t = 2.17, p-value = 0.082) or age at maturity (paired t-test; t = 0.349, p-value = 0.74) between males and females from the predator-free lakes and Karusputten (Table S2). Thus, we considered only combined males and females for subsequent analysis. Overall, age at-maturity for both sexes combined ranged from 2.6 years in Forkerudtjern to 4.0 years in Mottjernet (Table 2). Gonadosomatic index was highly variable among lakes and ranged from 1.86 in Brynitjernet to 16.09 in Øvresetetjern. Reproductive life span ranged from 3.1 years in Brynitjernet to 9.6 years in Stomperudtjern.

Gonadosomatic index and reproductive life span were positively correlated ($r = 0.79$, $p < 0.028$).

Relating predation risk with life-history traits

Predator gape size was positively correlated with crucian carp relative body depth ($r = 0.93$, $p < 0.001$) and negatively correlated with fish density ($r = -0.88$, $p < 0.001$). In absence of predators, fish populations consisted of abundant shallow-bodied individuals (Figure 3). By contrast, in presence of piscivores, catches were dominated by low densities of deep-bodied individuals, which were mostly well above predator gape size, especially in lakes with perch and pike. Growth traits L_∞ ($r = 0.86$, $p < 0.001$), L_1 ($r = 0.81$, $p < 0.001$), L_3 ($r = 0.89$, $p < 0.001$) and maximum life span ($r = 0.85$, $p < 0.001$) were all positively correlated with predator gape size, with the exception of the growth coefficient k ($r = -0.24$, $p = 0.39$; Figure 4a-e). Among reproductive traits, length at maturity showed positive correlation with gape size ($r = 0.82$ $p = 0.008$; Figure 4g), contrary to age at maturity ($r = -0.22$, $p = 0.60$; Figure 4h). Gonadosomatic index ($r = 0.65$, $p = 0.008$) and sex ratio ($r = 0.69$, $p = 0.004$) also increased significantly with gape size (Figure 4f, 4i). Reproductive life span ($r = 0.63$; $p = 0.096$) and mortality were not significantly correlated with predation risk ($r = -0.29$; $p = 0.42$). However, mortality analysis included mostly allopatric populations with highly variable values, while several high-predation lakes were excluded (Figure S1), which might have prevented us to detect a clear pattern.

DISCUSSION

Crucian carp population structure and life-history traits were clearly related to the presence or absence of piscivorous fish. As expected, fish achieved larger size, older age, and higher relative body depth with increasing predation risk. This size increment was evident at young age, especially in productive lakes with pike. Contrary to our predictions, populations had similar age at maturity and reproductive effort was increasing with predation risk. Crucian carp also showed small differences in growth between sexes. Predator-free lakes were largely dominated by females.

Young crucian carp attained larger body length with increasing predator gape size, showing clearly a higher growth rate during the first years of life in presence of predators. This suggests that fish quickly outgrew predator gape size to reach a size-refuge. However, the growth coefficient was not correlated to gape size and was generally low in lakes with piscivores. Indeed, the growth coefficient was inversely related to asymptotic length, probably because of increased time to reach very large maximum body sizes. Fish populations had also a larger life span with increasing predation risk. In predator-free lakes there could be two main constraints preventing fish from growing to older age: a food constrain and a seasonality constrain. Dense allopatric populations suffer high intraspecific competition and adult mortality, with a few fish reaching large sizes (Tonn et al., 1994). This food constraint might be lowered by the thinning of the populations in presence of increasingly efficient predators. Thus, predation can have an indirect positive effect on prey growth by reducing the density of prey population and releasing survivors from competition (Van Buskirk & Yurewicz, 1998).

These dynamics are expected to be highly dependent on resource availability (Day et al., 2002). Interestingly, in Lakes Posttjernet and Karussputten, crucian carp had the lowest length at age 1 and 3 compared to the other predator-lakes, including Småvanna, which had a similar fish community. These lakes had the lowest nutrient values, suggesting that growth might be limited by resource availability. By contrast, pike lakes were very productive and supported communities with different species. These lakes also presented a great proportion of complex vegetated littoral habitats, which may provide fish with a habitat refuge from predators. Resource and refuge availability may favor crucian carp body growth even after fish have outgrown predator gape size. Predator-free lakes with high productivity such as Forkerudtjern and Mottjernet supported dense crucian carp populations. In absence of predators, however, growth and reproductive traits were particularly variable and may depend on other factors in addition to resource availability. Lake structure is particularly important as small ponds at northern latitudes are subject to extreme seasonal changes, such as abrupt rising temperatures during summer and anoxic conditions during winter. Water temperature regulates crucian carp growth (Tarkan et al., 2011) and spawning (Aho & Holopainen, 2000), and interannual changes in temperatures likely have strong effects on life-history traits of fish living in small systems (Dembski et al., 2006). Moreover, while the main growth period for crucian carp is summer, in autumn fish need to build up reserves for overwintering (Piironen & Holopainen, 1986). Small crucian carp from allopatric populations have been observed to store a significantly higher proportion of glycogen reserves compared to large fish from multispecies communities (Vornanen et al., 2011). The liver was also the main glycogen store for small fish, while large fish used white muscle as main deposit. Fish from allopatric populations might invest more energy and time in building up winter reserves as they inhabit ponds with harsh environmental conditions. Moreover, energy reserve requirements are expected to vary greatly even among predator-free populations because of different lake structure, and thus oxygen availability during winter, with consequences on fish mortality, growth, and population dynamics (Fox & Keast, 1990).

Age at sexual maturity was variable among lakes, with values ranging between 3 and 4 years. These results were comparable to the values of populations from similar latitudes (Holopainen & Pitkänen, 1985), although studies on length and age at maturity of crucian carp are very rare in Fennoscandia. Contrary to our predictions, age at maturity was not increasing with predation risk. By contrast, fish reached length at maturity at larger sizes with predators, as a result of increased growth at young age. Gonadosomatic index values (GSI) of fish populations were increasing with predation risk. This variation may partly reflect differences in sampling time, as crucian carp is a batch spawner and the number of eggs might decrease after the first batch is released (Aho & Holopainen, 2000). However, previous studies found that large females from multispecies communities had higher gonadosomatic index, absolute and relative fecundity, and larger eggs, and proposed that reproductive effort increases with body size (Moisander, 1991; Holopainen et al., 1997a). The number of batches during each spawning season seems to depend on water temperature rather than predation risk or other environmental factors (Aho & Holopainen, 2000). Moreover, the gonadosomatic index was positively correlated with the reproductive life span. These results seem to contrast with life-history theory since a high reproductive effort should happen at the expense of growth or survival (Stearns, 1992). However, previous studies on crucian carp considered mainly large females that were probably already well outside of the predation window. Small fish from predator lakes may mature early but still invest more energy in growth than reproduction in order to reach a size refuge from predation. Then, they may slow down growth and allocate more energy to reproduction.

Moreover, we observed slight differences in growth between sexes. In some predator-lakes females had lower relative body depth compared to males, suggesting a potential higher investment in reproduction. Interestingly, the adult sex ratio was female-biased in lakes without predators and male-biased in lakes with predators. Since sexes did not differ in their age at maturity, we can exclude the effect of higher density of the sex that matures earlier. Sex ratio can be the result of differences in adult mortality (Arendt et al., 2014). Experimental studies showed that crucian carp females lose the typical predator avoidance behavior just before spawning (Lastein et al., 2008). This bolder behavior might increase predation risk for females but is displayed for a short time. On the contrary, females might be less active and take refuge in the littoral vegetation compared to males (Estlander & Nurminen, 2014), which also may have reduced their catchability. In lakes without predators, males had generally smaller sizes than females, suggesting that they might be subject to higher mortality early in life and females would eventually result more abundant.

Crucian carp is exceptionally vulnerable to predation (Tonn et al., 1991) and displays specific adaptations to elude predation risk (Nilsson & Brönmark, 2000). Our study shows that fish can achieve a size refuge by increasing both body depth and size, and the degree of growth is highly proportional to the gape size of the predators present in a lake. Growth is evident at young age when fish are most vulnerable to predation. Crucian carp allocate more energy to reproduction, grow older, and attain larger asymptotic sizes in presence of predators, which strongly suggests higher food availability in these lakes. Fish from predator lakes experience higher levels of resource availability as an indirect consequence of reduced intraspecific competition caused by predation. High-predation lakes are also highly productive systems with structural complexity, which may favor growth and reproductive allocation even after fish have outgrown predator gape size. Future studies should specifically address variation in reproductive effort with age in presence of predators. Moreover, crucian carp seem to decrease their activity levels, rather undergo risky behaviors, when exposed to piscivores (Holopainen et al., 1997b). Thus, fish may reduce foraging activity and the energy saved is then allocated to both growth and reproduction. This energy-saving mechanism is boosted by the effects of reduced intraspecific competition and increased favorable environmental conditions. On the contrary, high resource competition in allopatric populations will likely force individuals to active foraging to increase survival. High variability in life-history traits among populations suggests that biotic factors play a decisive role in regulating population dynamics of these lakes and should be better investigated.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

IdM.: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead). **KØ:** Conceptualization (equal); methodology (equal); resources (equal); writing-review & editing (supporting). **KKK:** Conceptualization (equal); formal analysis (supporting); methodology (equal); writing-review & editing (supporting). **ABSP:** Conceptualization (equal); project administration (lead); resources (lead); writing- review & editing (supporting).

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FIGURE LEGEND

Figure 1. Variation in relative body depth (RBD) between female (F) and male (M) crucian carp from the fifteen study lakes. Asterisks (*) denote significant differences ($p < 0.05$) between females and males. The dashed line separates lakes without predators (left side) from lakes with predators (right side).

Figure 2. Von Bertalanffy growth curves for crucian carp from 15 lakes without predator fish (first and second upper rows), with brown trout (second upper row), perch or perch and brown trout (second lower row), and pike and perch (first lower row). Shaded areas around the growth curves correspond to the 95% credibility intervals.

Figure 3. Body depth distribution of female (red) and male (blue) crucian carp from 15 lakes without predator fish (first and second upper rows), with brown trout (second upper row), perch or perch and brown trout (second lower row), and pike and perch (first lower row). Dashed lines indicate maximum predator gape size (GS; see Table 1).

Figure 4. Correlations between different maximum predator gape size and life-history traits of 15 populations of crucian carp from southern Norway. Life-history growth traits include (a) asymptotic length (L_∞), (b) growth coefficient (k), (c) length at age 1 (L_1), (d) length at age 3 (L_3) and (e) maximum life span (T_{max}). Reproductive traits include: (f) sex ratio (m:f), (g) length at maturity (L_m) and (h) age at maturity (A_m), and (i) gonadosomatic index (GSI). The shaded area corresponds to the 95% bootstrapped confidence interval.

FIGURES

Figure 1

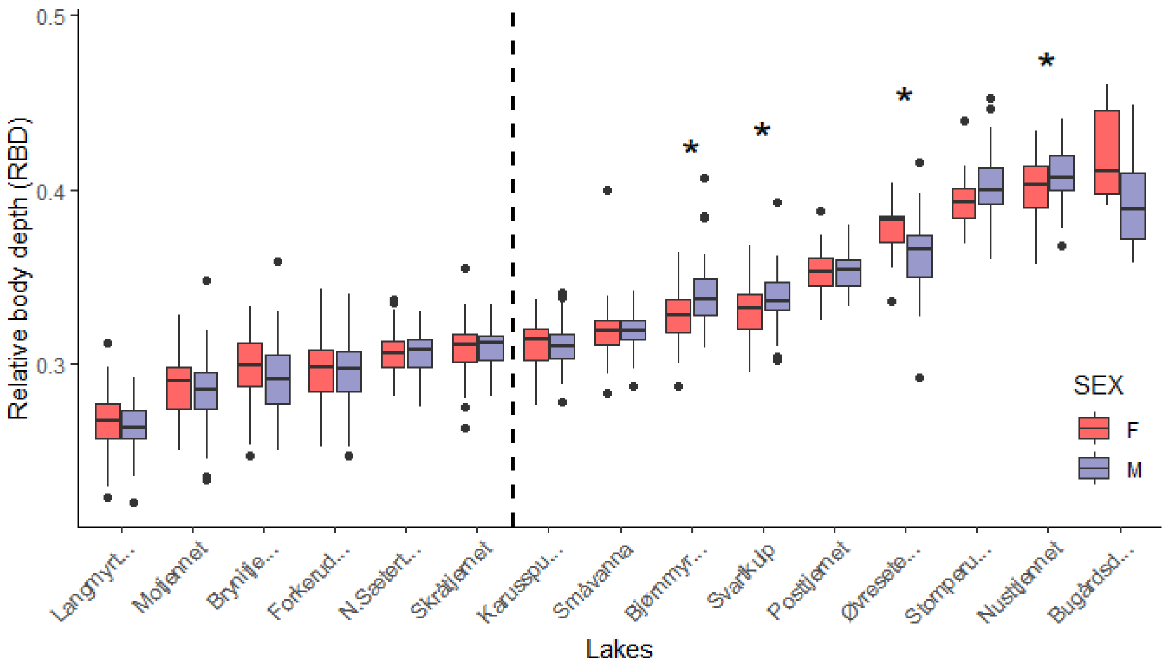


Figure 2

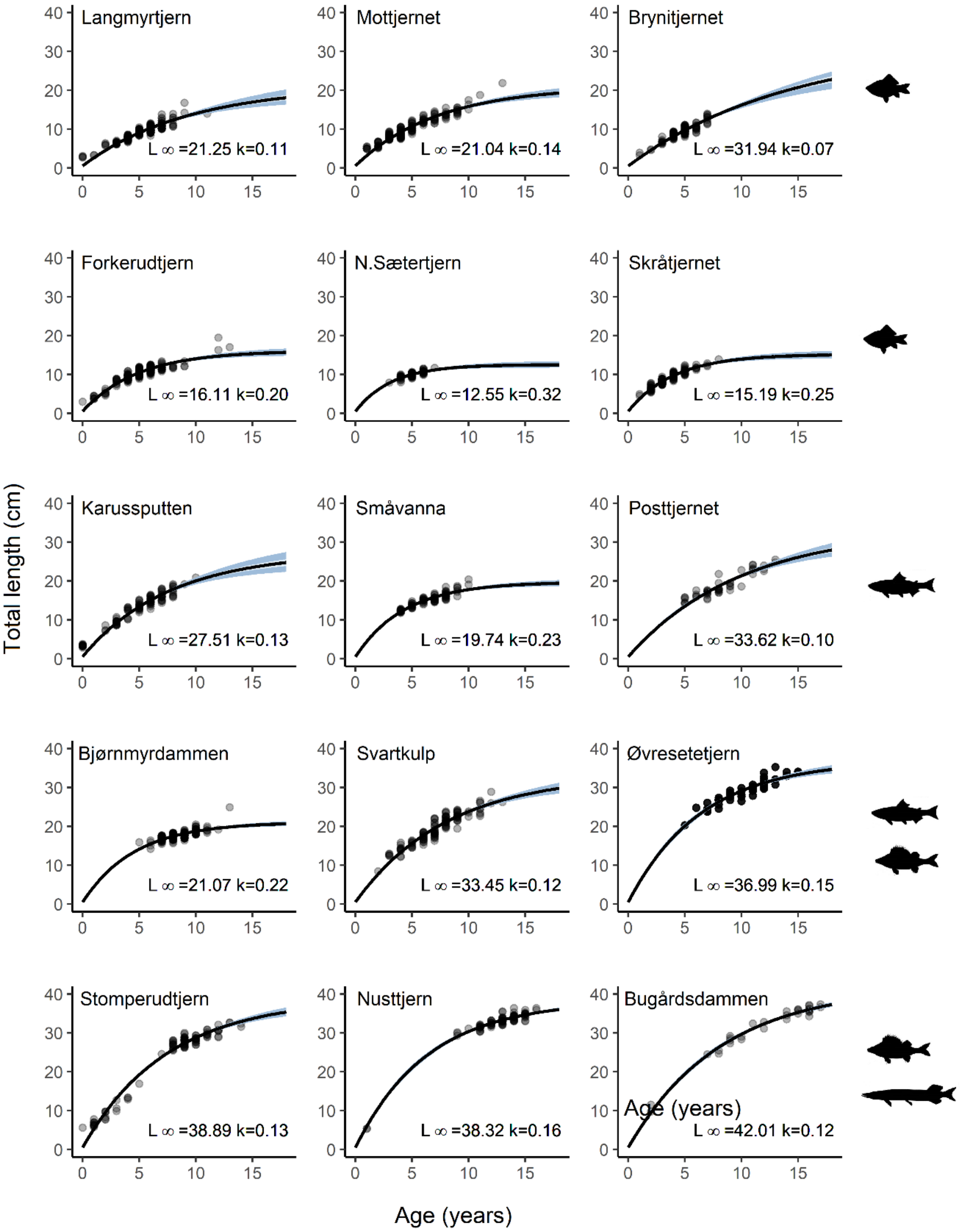

Langmyrtjern
L ∞ =21.25 k=0.11
Mottjernet
L ∞ =21.04 k=0.14
Brynitjernet
L ∞ =31.94 k=0.07
Forkerudtjern
L ∞ =16.11 k=0.20
N.Sætertjern
L ∞ =12.55 k=0.32
Skråtjernet
L ∞ =15.19 k=0.25
Karussputten
L ∞ =27.51 k=0.13
Småvanna
L ∞ =19.74 k=0.23
Posttjernet
L ∞ =33.62 k=0.10
Bjørnmyrdammen
L ∞ =21.07 k=0.22
Svartkulp
L ∞ =33.45 k=0.12
Øvresetetjern
L ∞ =36.99 k=0.15
Stomperudtjern
L ∞ =38.89 k=0.13
Nusttjern
L ∞ =38.32 k=0.16
Bugårdsdammen
Age (years)
L ∞ =42.01 k=0.12
Total length (cm)
Age (years)
0
10
20
30
40
0
5
10
15

Figure 3

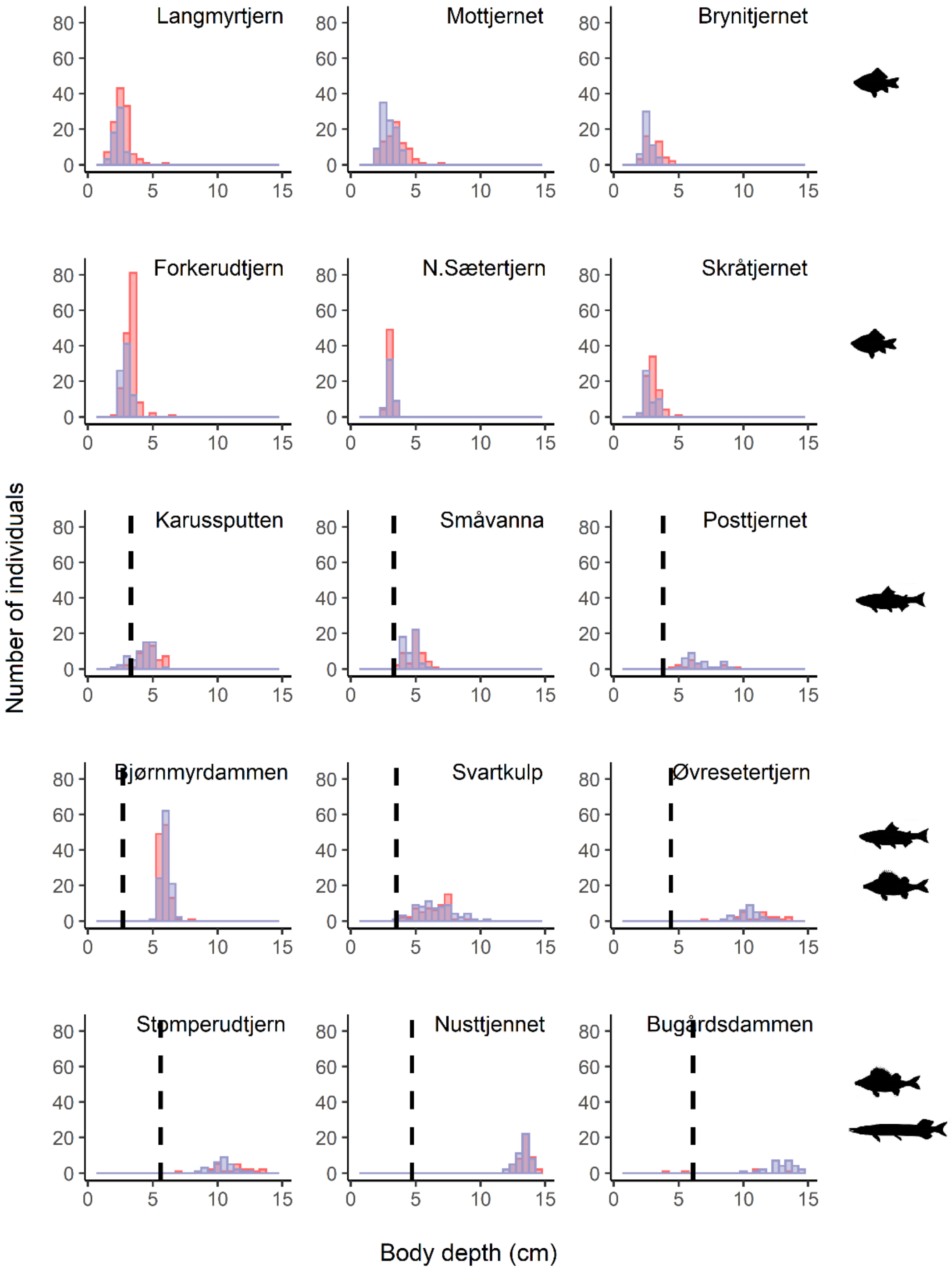
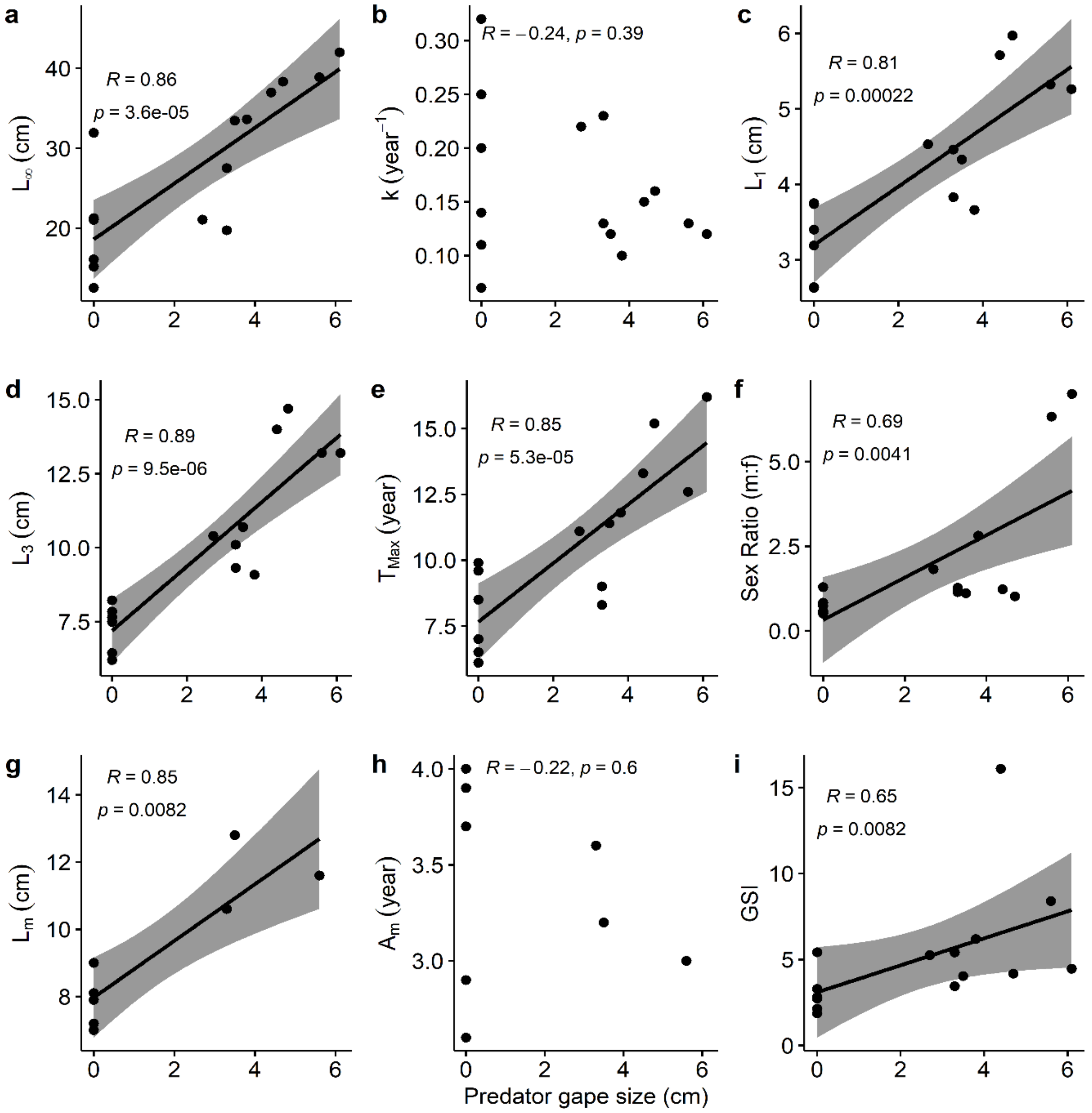

Langmyrtjern
Mottjernet
Brynitjernet
Forkerudtjern
N.Sætertjern
Skråtjernet
Karussputten
Småvanna
Posttjernet
Bjørnmyrdammen
Svartkulp
Øvresetertjern
Stomperudtjern
Nusttjennet
Bugårdsdammen
0
20
40
60
80
0
5
10
15
Number of individuals
Body depth (cm)

Figure 4


a
R = 0.86
p = 3.6e-05
L∞ (cm)
20
30
40
0
2
4
6
b
R = −0.24, p = 0.39
k (year⁻¹)
0.10
0.15
0.20
0.25
0.30
0
2
4
6
c
R = 0.81
p = 0.00022
L1 (cm)
3
4
5
6
0
2
4
6
d
R = 0.89
p = 9.5e-06
L3 (cm)
7.5
10.0
12.5
15.0
0
2
4
6
e
R = 0.85
p = 5.3e-05
TMax (year)
7.5
10.0
12.5
15.0
0
2
4
6
f
R = 0.69
p = 0.0041
Sex Ratio (m:f)
0.0
2.5
5.0
0
2
4
6
g
R = 0.85
p = 0.0082
Lm (cm)
8
10
12
14
0
2
4
6
h
R = −0.22, p = 0.6
Am (year)
3.0
3.5
4.0
0
2
4
6
Predator gape size (cm)
i
R = 0.65
p = 0.0082
GSI
0
5
10
15
0
2
4
6

TABLES

Table 1. Environmental characteristics of 15 study lakes. Abiotic variables include latitude (Lat), longitude (Long), lake area (Area), maximum depth (MaxD), total nitrogen (TotN), total phosphorus (TotP), total organic carbon (TOC). Maximum predator gape size (GS) and crucian carp density (CPUEcc) are also included.

Lake	**Predators**	**Lat (^{0}N)**	**Long (^{0}E)**	**Area (ha)**	**MaxD (m)**	**TotN (µg/L)**	**TotP (µg/L)**	**TOC (mg/L)**	**GS (cm)**	**CPUEcc (n·net^{-1}h^{-1})**
Nusttjennet	Pike Perch	60.28	11.66	11.00	1.5	1090	164	16.4	4.7	0.45
Bugårdsdammen	Pike Perch	59.13	10.19	5.04	2	980	54	9.5	6.1	0.10
Stomperudtjern	Pike Perch	59.32	11.40	3.85	1.5	1660	146	18.4	5.6	0.42
Øvresetertjern	Perch Trout	59.98	10.67	3.05	3.5	446	13	6.6	4.4	0.42
Posttjernet	Trout	61.08	11.33	1.72	11	312	8	9.7	3.8	0.19
Svartkulp	Perch Trout	59.98	10.74	5.80	10	550	13	9.9	3.5	0.30
Bjørnmyrdammen	Perch	60.18	11.98	2.10	3.5	672	26	6.5	2.7	2.11
Småvanna	Trout	59.80	10.31	0.50	3.8	616	14	10.1	3.3	1.03
Karussputten	Trout	60.02	10.66	0.25	4.6	361	9	5.4	3.3	0.99
Skråtjernet	-	60.20	11.14	0.88	12.0	431	15	5.3	-	1.83
N.Sætertjern	-	60.22	12.01	0.57	2.5	-	-	-	-	7.93
Forkerudstjern	-	60.45	12.08	1.24	2.2	1985	82	23.4	-	10.63
Brynitjernet	-	60.72	11.27	4.29	1.5	572	22	15.4	-	4.08
Motjennet	-	60.23	12.11	0.94	11.3	688	23	11.2	-	7.80
Langmyrtjern	-	59.97	10.75	0.30	5	702	20	14.2	-	2.07

Table 2. Life-history characteristics of 15 populations of crucian carp from southern Norway. Mean Fulton's condition factor (K_{ConF}) and relative body depth (RBD). Total number of individuals used for length-at-age estimation (n). Mean asymptotic length (L_∞), growth coefficient (k), length at age 1 (L_1) and 3 (L_3) estimated from the von Bertalanffy function, where values in brackets are the SD of the respective parameters from their posterior distributions. Maximum life span (T_{Max}). Mean length (L_M) and age (A_M) at maturity, where numbers in parentheses are the 95% Bootstrapping confidence intervals. Reproductive life span (RT). Sample distribution and size prevented some of the maturity calculations and were marked with -. Sex ratio (males:females) and mean gonadosomatic index (GSI) for mature females. All lengths (L) refer to total body length (TL) in cm. T_{Max}, A_M, and RT are in years and k is in year^{-1}.

Lakes	**Predators**	**K_{ConF}**	**RBD**	**n**	**L_∞**	**k**	**L_1**	**L_3**	**T_{Max}**	**SexR**	**L_M**	**A_M**	**RT**	**GSI**
Nusttjernet	Pike, Perch	2.35	0.40	86	38.3 (0.6)	0.16 (0.01)	6.0	14.7	15.2	1.02	-	-	-	4.18
Bugårdsdammen	Pike, Perch	2.31	0.40	31	42.0 (0.9)	0.12 (0.01)	5.5	13.2	16.2	7.00	-	-	-	4.46
Stomperudtjern	Pike, Perch	2.31	0.40	99	38.9 (1.2)	0.13 (0.01)	5.3	13.2	12.6	6.33	11.6 (11.3-11.9)	3.0 (2.5-3.5)	9.6	8.39
Øvresetetjern	Perch, Trout	2.22	0.37	49	37.0 (1.0)	0.15 (0.01)	5.7	14.0	13.3	1.23	-	-	-	16.09
Posttjernet	Trout	2.03	0.35	47	33.6 (2.5)	0.10 (0.01)	3.7	9.1	11.8	2.81	-	-	-	6.19
Svartkulp	Perch, Trout	1.85	0.33	124	33.5 (1.5)	0.12 (0.01)	4.3	10.7	11.4	1.11	12.8 (11.6-13.8)	3.2 (2.2-3.9)	8.2	4.03
Bjørnmyrdammen	Perch	1.82	0.33	179	21.1 (0.4)	0.22 (0.01)	4.5	10.4	11.1	1.82	-	-	-	5.25
Småvanna	Trout	1.77	0.32	95	19.7 (0.5)	0.23 (0.01)	4.5	10.1	9	1.14	-	<4	-	5.40
Karussputten	Trout	1.67	0.31	112	27.5 (2.4)	0.13 (0.02)	3.8	9.2	8.3	1.27	10.6 (10.0-11.3)	3.6 (3.2-3.9)	4.7	3.44
Skråtjernet	-	1.63	0.31	143	15.2 (0.5)	0.25 (0.02)	3.7	8.2	6.5	0.58	7.9 (7.7-8.1)	2.9 (2.8-3.0)	3.6	2.13
N.Sætertjern	-	1.57	0.31	105	12.6 (0.5)	0.32 (0.03)	3.8	7.8	6.1	0.74	-	-	-	5.42
Forkerudtjern	-	1.61	0.30	192	16.1 (0.6)	0.20 (0.01)	3.4	7.7	9.6	0.55	7.0 (6.5-7.7)	2.6 (2.3-3.0)	7	2.73
Brynitjernet	-	1.75	0.30	107	31.9 (4.1)	0.07 (0.01)	2.6	6.4	7	0.83	8.1 (7.5-8.6)	3.9 (3.4-4.3)	3.1	1.86
Mottjernet	-	1.43	0.29	185	21.0 (1.0)	0.14 (0.01)	3.2	7.5	9.9	1.29	9.0 (8.8-9.2)	4.0 (3.8-4.2)	5.9	3.29
Langmyrtjern	-	1.36	0.27	179	21.3 (2.3)	0.11 (0.02)	2.6	6.2	8.8	0.51	7.2 (7.0-7.4)	3.7 (3.5-4.0)	4.8	2.81

SUPPLEMENTARY MATERIAL

Table S1. Equations of the candidate growth models used in leave-one-out-cross-validation (LOOCV) procedure, where L_a is the length-at-age a, L_0 is the length-at-birth, L_∞ is the asymptotic length at which growth is zero, and k is the growth completion parameter.

Model	***Growth function***
Von Bertalanffy	$L_a = L_\infty - (L_\infty - L_0)e^{-ka}$
Logistic function	$L_a = (L_\infty L_0 e^{ka})/(L_\infty + L_0 (e^{ka}-1)$
Gompertz function	$L_a = L_0 e^{(log(L_\infty / L_0)(1- e^{-ka}))}$

Table S2. Reproductive traits of male and female crucian carp from the 15 study lakes. Total number of individuals used for length at-age-estimation (n). Mean asymptotic length (L_∞), growth coefficient (k), estimated from the von Bertalanffy function, where the values in brackets are the SD of the respective parameters from their posterior distributions. Mean length (L_M) and age (A_M) at maturity, where the values in parentheses are the 95% Bootstrapping confidence intervals. Sample distribution and size prevented some of the maturity calculations and were marked with -.

Lakes	**Predators**	**Females**					**Males**				
		n	**L_∞**	**k**	**L_M**	**A_M**	**n**	**L_∞**	**k**	**L_M**	**A_M**
Nusttjernet	Pike, Perch	42	40.3 (1.0)	0.14 (0.01)	-	-	46	36.5 (0.5)	0.18 (0.01)	-	-
Bugårdsdammen	Pike, Perch	4	37.6 (3.2)	0.16 (0.04)	-	-	26	42.2 (0.9)	0.12 (0.01)	-	-
Stomperudtjern	Pike, Perch	26	39.3 (2.5)	0.13 (0.02)	-	-	87	38.3 (1.2)	0.14 (0.01)	-	-
Øvresetetjern	Perch, Trout	21	38.5 (1.4)	0.14 (0.01)	-	-	28	34.8 (1.3)	0.18 (0.02)	-	-
Svartkulp	Trout	55	35.6 (2.5)	0.11 (0.01)	-	-	69	33.3 (1.8)	0.13 (0.01)	-	-
Bjørnmyrdammen	Perch, Trout	94	22.2 (0.9)	0.19 (0.02)	-	-	85	20.3 (0.4)	0.24 (0.02)	-	-
Posttjernet	Perch	18	34.4 (3.4)	0.10 (0.02)	-	-	29	35.6 (3.0)	0.09 (0.01)	-	-
Småvanna	Trout	46	21.9 (1.5)	0.19 (0.02)		<4	49	18.7 (0.5)	0.26 (0.02)		<4
Karussputten	Trout	49	30.9 (3.6)	0.11 (0.02)	10.6 (9.4-12.0)	3.6 (3.0-4.1)	46	33.7 (4.0)	0.10 (0.02)	10.6 (10.0-11.3)	3.8 (3.3-4.1)
Forkerudtjern	-	128	16.9 (0.8)	0.19 (0.02)	7.4 (6.8-8.1)	2.9 (2.6-3.3)	69	13.2 (0.7)	0.29 (0.03)	7.4 (6.8-8.0)	2.8 (2.5-3.1)
Mottjernet	-	120	23.1 (1.5)	0.12 (0.01)	9.5 (9.1-10.0)	4.3 (4.0-4.6)	126	16.2 (0.9)	0.21 (0.02)	8.9 (8.7-9.1)	4.1 (3.8-4.3)
Langmyrtjern	-	102	25.1 (3.8)	0.09 (0.02)	7.3 (7.0-7.7)	3.7 (3.5-4.0)	56	29.47 (5.75)	0.07 (0.02)	7.3 (7.0-7.6)	4.0 (3.9-4.0)
N.Sætertjern	-	59	12.8 (0.7)	0.30 (0.04)	-	-	46	27.70 (0.81)	0.20 (0.06)	-	-
Skråtjernet	-	76	15.7 (0.8)	0.24 (0.02)	8.2 (7.9-8.4)	3.0 (2.9-3.2)	45	15.8 (1.1)	0.24 (0.02)	7.8 (7.6-8.2)	2.9 (2.8-3.1)
Brynitjernet	-	61	34.1 (3.9)	0.07 (0.01)	8.7 (8.0-9.3)	4.2 (3.9-4.6)	59	33.6 (4.4)	0.07 (0.01)	8.2 (7.5-8.8)	3.9 (3.2-4.5)

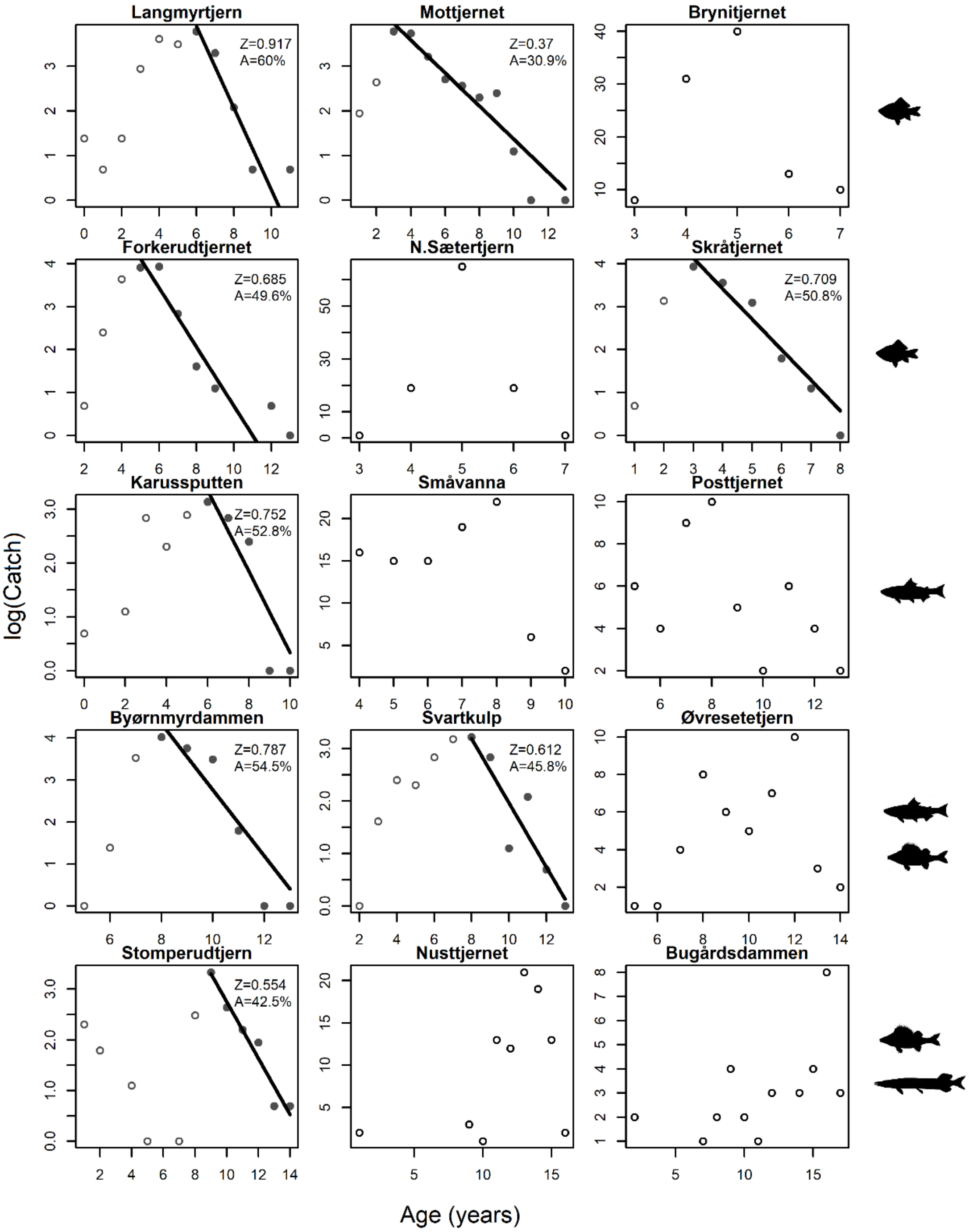


Figure S1. Scatterplots of the natural logarithm of catch at age for crucian carp from 15 lakes without predator fish (first and second upper rows), with brown trout (second upper row), perch or perch and brown trout (second lower row), and pike and perch (first lower row). The solid circles represent the descending limb of the catch-curve and were used to estimate total annual mortality rate (*A*) and instantaneous mortality rate (*Z*).

Inland Norway
University of
Applied Sciences

The crucian carp *(Carassius carassius)* is a well-studied model organism in regard to phenotypic plasticity in anti-predator defenses, as it develops a deep body shape to decrease vulnerability to predation. Very few studies, however, have investigated the mechanisms underlying the defense response of crucian carp in a broader ecological and environmental context. This work explored the effects of predation risk on crucian carp morphology, resource use, and life-history traits. We considered fifteen small lakes from southern Norway along a natural gradient of predation risk: no predators or brown trout, perch, or pike. Crucian carp showed a fine-tuned morphological defense response against increasingly efficient predators. Predation risk determined an increase in crucian carp body depth, rapid juvenile growth, larger lifespans, and higher reproductive investment. Predation pressure likely reduced crucian carp density, relaxing intraspecific competition and supporting individual growth. Higher productivity and habitat complexity associated with high-predation lakes and plastic diet preferences may also have favored energy allocation to growth and reproduction. Thus, the expression of the defense response in crucian carp was likely triggered by the combined effects of predation risk and resource availability. Investigating how different environmental factors affect inducible defense responses can give new insights into the evolution of phenotypic plasticity. Finally, the understanding of these processes is important as current human activities can have strong impacts on ecological interactions in freshwater ecosystems.