Defence on Demand

A physiological perspective on phenotypic plasticity in anti-predator traits

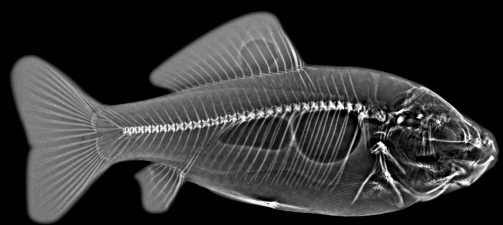
Vinterstare, Jerker

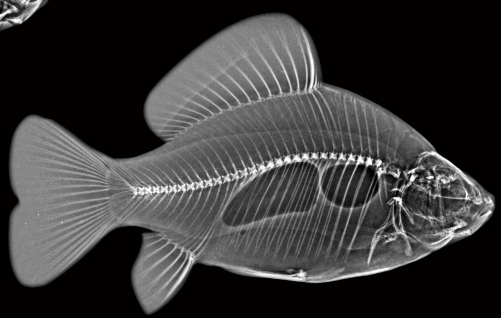
2021

Defence on Demand

A physiological perspective on phenotypic plasticity in anti-predator traits

JERKER VINTERSTARE
DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY





Defence on Demand

A physiological perspective on phenotypic plasticity in anti-predator traits

Jerker Vinterstare

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Science, Lund University, Sweden.
To be defended in the Blue Hall, Ecology Building, Sölvegatan 37, Lund,
Sweden on Friday 19th of March, 2021 at 13.00.

Faculty opponent
Professor Neil Metcalfe
Institute of Biodiversity Animal Health & Comparative Medicine
University of Glasgow

Organization LUND UNIVERSITY	Document name: Doctoral Dissertation
	Date of issue: March 19 2021.
Author: Jerker Vinterstare	Sponsoring organization

Title and subtitle
Defence on Demand A physiological perspective on phenotypic plasticity in anti-predator traits

Abstract
Almost all species face some degree of predation risk, and, hence, evolution has produced a plethora of anti-predator defences. However, anti-predator strategies require resources, and the prevailing risk of becoming prey is influenced by many factors and rarely constant across time and space. Evolution has therefore favoured the development of phenotypic plasticity in anti-predator defences. The capacity of a single genotype to fine-tune its phenotype according to the prevailing risk of predation results in a closer phenotype-environment match in the mercurial environments of the natural world. Interest in the ecology and evolution of inducible defences has progressed the development of its theoretical underpinnings, along with empirical tests of theoretical predictions. Inter-individual differences in the expression of inducible traits are nowadays understood ubiquitous, and this intriguing variation holds possibility to bridge our current knowledge gap on the proximate, physiological mechanisms underlying inducible morphological defence regulation.

In this thesis, I address unanswered questions on the proximate, physiological processes behind phenotypic plasticity in morphological defences. I first examine classic resource-allocation trade-offs to search for hidden physiological costs coupled with perceived predation risk and investment into a morphological defence. I further employ a recent hypothesis of physiological stress being the mechanism driving morphological defence regulation. To test my predictions, I have used a well-established model system for the study of inducible morphological defences, the crucian carp (*Carassius carassius*).

I show that innate immune functions are altered by predator exposure, and that the defence against pathogens is correlated with the morphological defence against larger-sized enemies (predators). Second, from a series of laboratory experiments and field studies, I demonstrate support for the hypothesis of stress physiology being involved in plastic defence expression, and that crucian carp display clear sexual dimorphism in the anti-predator phenotype. I suggest that this is due to sex-specific trade-off dynamics, where females invest more into reproduction, resulting in less resources for anti-predator protection. Finally, I present novel trait changes (changes in body colour, diel activity and eye morphology) in response to non-lethal predation risk that may act in synergy to produce an integrated anti-predator phenotype.

In summary, my results illustrate a potential causality from the vertebrate stress axis to the regulation of morphological defence expression. Further, my thesis highlights variation and complexity on the route to producing optimal anti-predator phenotypes under competing demands from other interests.

Key words: Phenotypic plasticity, inducible defences, stress physiology, cortisol, immune function, trade-offs, sexual dimorphism, crucian carp, visual ecology, predator-prey interactions, POMC (pro-opiomelanocortin), colour change, eco-immunology

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language** Eng
ISBN: 978-91-7895-755-2 (print), 978-91-7895-756-9 (pdf)		
Recipient"s notes	**Number of pages** 202	Price
	Security classification	

Signature  Date 2021-02-09

Defence on Demand

A physiological perspective on phenotypic plasticity in anti-predator traits

Jerker Vinterstare

LUND
UNIVERSITY

Cover photo by Jerker Vinterstare (X-ray crucian carp) and Jörgen Wiklund (pike)

Faculty of Science

Department of Biology

ISBN 978-91-7895-755-2 (print)

ISBN 978-91-7895-756-9 (pdf)

Printed in Sweden by Media-Tryck, Lund University

Lund 2021

For Vidar and Sigrid

There are these two young fish swimming along and they happen to meet an older fish swimming the other way, who nods at them and says "Morning, boys. How's the water?" And the two young fish swim on for a bit, and then eventually one of them looks over at the other and goes "What the hell is water?"

David Foster Wallace - 2005

Table of Contents

List of papers

In this thesis the papers are referred to by the following roman numerals

I. Vinterstare, J, Hegemann, A, Nilsson, PA, Hulthén, K & Brönmark, C 2019, Defence versus defence: Are crucian carp trading off immune function against predator-induced morphology?, *Journal of Animal Ecology*, 88: 1510-1521.

II. Vinterstare, J, Hulthén, K, Nilsson, PA, Nilsson Sköld, H & Brönmark, C 2020, Experimental manipulation of perceived predation risk and cortisol generates contrasting trait trajectories in plastic crucian carp, *Journal of experimental biology*, vol. 223, jeb213611.

III. Vinterstare, J, Brönmark, C, Nilsson, PA, Langerhans, RB, Berglund, O, Örjes, J, Brodin, T, Fick, J, & Hulthén, K. Anti-predator phenotype in crucian carp altered by a psychoactive drug. *Submitted*.

IV. Vinterstare, J, Hulthén, K, Nilsson, PA, Langerhans, RB, Chauhan, P, Hansson, B, & Brönmark, C. Sex matters: different expressions of inducible morphological defence and stress genes between sexes in crucian carp. *Manuscript*.

V. Vinterstare, J, Hulthén, K, Nilsson, DE, Nilsson, PA & Brönmark, C 2020, More than meets the eye: Predator-induced pupil size plasticity in a teleost fish, *Journal of Animal Ecology*, 89:2258-2267.

Author contributions

I. Defence versus defence: Are crucian carp trading off immune function against predator-induced morphology?

All authors contributed to the idea and design of the study. JV performed the experiment; AH carried out the immunological assays; JV carried out the statistical analyses and prepared the figures and tables with contributions from AH and PAN. The first draft of the manuscript was written by JV who led the writing and revision process together with AH. PAN, KH and CB contributed substantially to the final manuscript.

II. Experimental manipulation of perceived predation risk and cortisol generates contrasting trait trajectories in plastic crucian carp.

CB had the original idea and designed the study with help from JV, KH and PAN. JV performed the experiment with help from KH and CB. HNS did the scale analyses. JV carried out the statistical analyses and prepared the figures and tables with contributions from PAN, KH and CB. The first draft of the manuscript was written by JV with help from KH. All authors contributed significantly to the final version of the manuscript.

III. Anti-predator phenotype in crucian carp altered by a psychoactive drug.

CB and KH conceived the study together with OB and PAN. KH, JV and JÖ performed the laboratory experiment including the behavioural tests and water sampling. JV collected blood plasma samples which JF and TB analysed along with the water samples. KH and JV extracted morphological measurements from digital photographs and RBL performed the statistical analyses and produced the figures and tables together with JV. The first draft of the manuscript was written by JV and CB. All authors contributed significantly to the final version.

IV. Sex matters: different expressions of inducible morphological defence and stress genes between sexes in crucian carp.

JV developed the original idea and designed the study. JV performed the laboratory experiment and collected all field data. JV extracted morphological measurements from digital photographs and RBL performed the morphometric analyses and produced the figures and tables together with JV. PC did the bioinformatics. JV led the writing and revisions and KH, RBL, PAN, PC, BH and CB contributed substantially to the final manuscript.

V. More than meets the eye: Predator-induced pupil size plasticity in a teleost fish.

JV developed the original idea and designed the study together with CB. JV performed the experiments; DEN did the modelling of visual range; JV and DEN performed the statistical analyses and prepared figures and tables. JV led the writing and revisions and KH, DEN, PAN and CB contributed substantially to the final manuscript.

List of Authors: Jerker Vinterstare (JV), Christer Brönmark (CB), Kaj Hulthén (KH), P. Anders Nilsson (PAN), Arne Hegemann (AH), Dan-E Nilsson (DEN), Helen Nilsson Sköld (HNS), R. Brian Langerhans (RBL), Olof Berglund (OB), Jennie Örjes (JÖ), Thomas Brodin (TB), Jerker Fick (JF), Pallavi Chauhan (PC), Bengt Hansson (BH).

Abstract

Almost all species face some degree of predation risk, and, hence, evolution has produced a plethora of anti-predator defences. However, anti-predator strategies require resources, and the prevailing risk of becoming prey is influenced by many factors and rarely constant across time and space. Evolution has therefore favoured the development of phenotypic plasticity in anti-predator defences. The capacity of a single genotype to fine-tune its phenotype according to the prevailing risk of predation results in a closer phenotype-environment match in the mercurial environments of the natural world. Interest in the ecology and evolution of inducible defences has progressed the development of its theoretical underpinnings, along with empirical tests of theoretical predictions. Inter-individual differences in the expression of inducible traits are nowadays understood ubiquitous, and this intriguing variation holds possibility to bridge our current knowledge gap on the proximate, physiological mechanisms underlying inducible morphological defence regulation.

In this thesis, I address unanswered questions on the proximate, physiological processes behind phenotypic plasticity in morphological defences. I first examine classic resource-allocation trade-offs to search for hidden physiological costs coupled with perceived predation risk and investment into a morphological defence. I further employ a recent hypothesis of physiological stress being the mechanism driving morphological defence regulation. To test my predictions, I have used a well-established model system for the study of inducible morphological defences, the crucian carp (*Carassius carassius*).

I show that innate immune functions are altered by predator exposure, and that the defence against pathogens is correlated with the morphological defence against larger-sized enemies (predators). Second, from a series of laboratory experiments and field studies, I demonstrate support for the hypothesis of stress physiology being involved in plastic defence expression, and that crucian carp display clear sexual dimorphism in the anti-predator phenotype. I suggest that this is due to sex-specific trade-off dynamics, where females invest more into reproduction, resulting in less resources for anti-predator protection. Finally, I present novel trait changes (changes in body colour, diel activity and eye morphology) in response to non-lethal predation risk that may act in synergy to produce an integrated anti-predator phenotype.

In summary, my results illustrate a potential causality from the vertebrate stress axis to the regulation of morphological defence expression. Further, my thesis highlights variation and complexity on the route to producing optimal anti-predator phenotypes under competing demands from other interests.

Svensk sammanfattning

Majoriteten av alla organismer lever ständigt med risken att bli föda åt någon annan och det är därför, enligt ordets rätta bemärkelse livsviktigt att kunna försvara sig mot predatorer. Ett dåligt försvar kan innebära döden och i ett ögonblick omöjliggöra all framtida reproduktion och således reducera det vi alla i slutändan slåss om – andelen genvarianter i framtida genpool. Den här boken handlar om just detta, om försvarsstrategier mot predation. Den handlar om hur iögonfallande yttre försvar kan slås av och på vid behov, den utforskar det fysiologiska maskineri som sätter igång och upprätthåller uttrycket av kroppsliga försvar samt vilka för- och nackdelar ett sådant system kan medföra. Dessutom belyser boken en rad fascinerande försvarsstrategier vilka föreslås interagera i syfte att producera ett så optimalt försvar som möjligt. Nedan följer först en beskrivning av bokens huvudrollsinnehavare följt av en generell bakgrund till bokens syfte som per automatik återger vad jag sysslat med under de senaste 5.5 åren samt vad jag under denna tid funnit och inte funnit.

Okej, bokens huvudroll innehas av ingen mindre än den välstuderade fiskarten ruda. En allmänt förekommande art i norra Europas sötvatten. Varför just rudan stal hela scenen är på grund av artens minst sagt extrema överlevnadsstrategier. Nedgrävd i de syrefria sedimenten övervintrar rudan i bottenfrusna sjöar tills vårens fotosyntes återigen förser vattnet med biologiskt nödvändig syre. Övriga arter, vilka dog efter bara några minuter av vinterns syrebrist, kan då börja återkolonisera vattenmassorna. Rudan må hantera syrebrist bättre än de flesta, men i sin ursprungliga form är de ganska värdelösa på att undkomma hungriga rovfiskar. Det är här berättelsen tar vid, när rovfiskar såsom gädda och abborre gör entré, det är nämligen då bokens huvudkaraktär uppvisar en annan extrem anpassning för att maximera chansen till överlevnad. När doften av fara och död sprider sig i vattenmassorna börjar de likt en karaktär i Transformers förändra sitt yttre. Från att som andra fiskar ha tillväxt främst längs det horisontella planet börjar nu en vertikal tillväxt ta vid, ett skifte i tillväxtriktning som i slutändan generar en form (morfologi) som är avsevärt mer svårhanterligt för rovfiskar vilka är begränsade storleken på sina gap. Den nya höga kroppsformen leder dessutom till bättre accelerationsförmåga, vilket är fördelaktigt när man exempelvis måste fly undan en projektil till attackerande gädda. Denna kroppsdimorfism som rudan uppvisar som svar på närvaro respektive frånvaro av rovfisk är så pass distinkt att man

historiskt separerade de båda formerna i två skilda arter. Även om fenomenet i sig varit känt sedan början av 1990-talet så har vi än idag ingen mekanistisk förståelse för hur denna kroppsförändring regleras utifrån ett fysiologiskt perspektiv. Den här frågan är på flera sätt bokens piedestal och jag återkommer därför till detta senare, men först en kort bakgrund till bokens syfte.

Predation d.v.s. interaktionen mellan minst två individer där den ena (predatorn) livnär sig på energin som är bunden i den andra (bytet) är tveklöst en av de mest primära dödsorsakerna i naturen. Effekterna av predation är mångfacetterade och sträcker sig från ekosystemnivå till selektion av specifika gener. Via direkt reglering av bytespopulationer innehar predation en betydande funktion i modern ekologi och av samma anledning har predatorer en elementär roll i det naturliga urvalet av organismer. För att maximera överlevnad i en värld full av hungriga fiender har årmiljoner av evolution genererat den ena spektakulära försvarsstrategin efter den andra. Tittar man efter så kan man skönja att naturen är full av taggar, sköldar, färger för fara, färger för kamouflage och beteenden så som att fly eller fäkta, gömma sig eller spela död. Då olika miljöer och predatorer skiljer sig åt är risken för ett byte sällan homogen, vare sig mellan arter och populationer eller mellan individer av samma population. Här är tid en viktig aspekt att ta hänsyn till eftersom allt förändras över tid och så även den lokala risken för predation. Då alla försvarsstrategier är associerade med kostnader är det bästa försvaret direkt beroende av rådande förhållande.

I miljöer där risken för predation är relativt konstant och således förutsägbar kan man förvänta sig ett proaktivt agerande d.v.s. bytets respons kan förväntas äga rum innan en akut fara uppstår. I mer heterogena miljöer, där predationsrisken varierar i tid och rum bör man istället förvänta sig ett mer reaktivt agerande d.v.s. bytets respons kan förväntas ske när den akuta faran initierats. Som nämnts ovan medför försvarsstrategier, precis som andra biologiska processer, kostnader. Undantaget den energiinvestering som processen att producera och upprätthålla ett kroppsligt (morfologiskt) försvar innehar så kan proaktiva och reaktiva strategier förväntas medföra indirekta kostnader. Kostnader med proaktiva anti-predator strategier är ofta näringsrelaterade, som att tvingas uppehålla sig på betesmarker av lägre kvalité eller att i grupp behöva dela maten på fler magar medan reaktiva strategier i regel sätter igång den neuroendokrina stressaxeln vilket medför förhöjda nivåer av stresshormoner, en respons som är energimässigt kostsam. För att minimera dessa kostnader kan försvar mot predation, likt andra biologiska egenskaper antingen vara permanenta eller föränderliga under individens livstid. Permanenta försvar är egenskaper vilka över tid blivit genetiskt fixerade i populationen och således uttryckta i fenotypen oavsett rådande risksituation. I kontrast till detta finner vi termen ”fenotypisk plasticitet” som beskriver en genotyp som kan uttrycka olika fenotyper beroende av miljön som den för stunden existerar i.

Med ordet ”fenotyp” menas här alla egenskaper som hos en organism är observerbara, från utseende (morfologi) till fysiologiska och beteendemässiga karaktärer. Per definition förstår man då snabbt att alla organismer är mer eller mindre plastiska. En hare går när räven sover och springer när räven attackerar, människor är experter på att plastiskt förändra beteendet utefter den sociala kontext som råder och vi nordbor är, på en helt annan tidsskala för plasticitet, generellt blekare under vintern än sommaren. En del former av plastiska försvar har vi god förståelse för medan andra är mindre välstuderade. Ett utrönt exempel är just människans inducerbara försvar mot solens UV-strålning och kanske är det inte så stor skillnad på en sol och en gädda? - ett märkligt påstående som jag nu måste försöka bena ut.

Vi vet att solens UV-strålning varierar i intensitet över året. Vi vet att strålningen är betydligt intensivare på sommaren än vintern och vi vet att strålningen är skadlig i den bemärkelse att den kan orsaka mutationer som i värsta fall dödar oss. Detta förklarar varför vi nordbor är bleka om vintern och bruna om sommaren. Den ultimata förklaringen finner vi i pigmentet melanin. Ett pigment som skyddar vår hudkostym via en kemisk process som omvandlar skadlig UV-strålning till värme. När vår hud träffas av solens UV-strålar så ökar vår melaninproduktion – vi blir brunare. På så vis regleras försvaret utefter aktuellt behov och vi undviker onödiga energikostnader som annars är associerade med melaninproduktion. Den proximata förklaringen för detta inducerbara försvar finner vi i uttrycket av en enda gen, den så kallade POMC-genen (pro-opoimelanocortin). Denna gen producerar en lång kedja av aminosyror som sedan klyvs till korta peptider med olika roller, häribland att stimulera produktionen av melanin. Det häftiga är att genuttrycket regleras av solen. Låt oss nu tänka oss att vi byter ut solen mot en gädda och människan mot en ruda. För att relatera till solbrännan kan en intressant egenskap hos POMC-genen noteras. Som redan nämnts kodar genen för ett protein som kan klyvas till flera olika biologiskt aktiva peptider. Sol-exponering leder till produktion av den melaninstimulerande peptiden α-MSH. I hypofysens framlob producerar POMC i stället ACTH, ett hormon vars huvudfunktion är att stimulera produktionen och frisättningen av så kallade glukokortikoider från binjurarna d.v.s. stresshormoner likt kortisol. POMC har alltså en nyckelroll i ryggradsdjurens stressaxel. Det intressanta i detta sammanhang är att just ökade nivåer av stresshormoner nyligen visat sig ha en regulatorisk funktion i beteenden vid försvar mot rovdjur. POMC kan således beskrivas som en universell komponent i stresshantering genom reglering av yttre försvarskaraktärer. Denna pigmenteringsförändring sker förstås på en helt annan och mycket snabbare tidsskala, sekunder eller mindre, jämfört med människans säsongsvariation i pigmentering. Spoiler alert! I artikel II visar jag att rudor får en avsevärt mörkare kroppsfärg när de ser och känner doften av en gädda. Gäddor har förvisso ingen skadlig UV-strålning (såvitt jag vet), så det adaptiva värdet

för en ruda att bli mörk kan diskuteras, vilket jag också gör (se nedan), men POMC-genen i sig vet inte orsaken utan reagerar på stress, vilket gör att solen och en gädda trots allt inte är så olika. Nåväl, nu byter vi ut solen till fördel för predatorer och solbrännan till fördel för andra kroppsliga försvar - vilka fungerar bättre mot ett tandfyllt gap än mot UV-strålning.

I en serie experiment (artikel II, III IV) har jag utforskat det fysiologiska maskineri som slår av och på rudans morfologiska försvar d.v.s. ”knappen” som skiftar tillväxten från horisontell till vertikal. Som jag varit inne på tidigare så har jag initialt arbetat utefter en hypotes om att predationsrisk gör byten stressade och att den stressen kan tänkas fungera som ett regulatoriskt maskineri för morfologiska försvar mot rovdjur. Vi vet att evolutionen främjar plasticitet i försvarskaraktärer i miljöer där predationsrisken varierar i tid och/eller rum. Det är således rimligt att anta att organismer i sådana miljöer har en annan anpassningsförmåga till kronisk stress än vad exempelvis zebror på savannen har, där lejon och hyenor alltid lurar. Jag har därför arbetat utefter en misstanke om att fysiologisk stress kan ha fått sekundära effekter hos bytesdjur som under livets gång upplever perioder av låg och hög predationsrisk d.v.s. att driva och upprätthålla kroppsliga försvar tills det att faran är över. Denna hypotes har på senare tid fått stöd då kortikosteron, en nära molekylsläkting till mer välkända kortisol, visat sig driva uttrycket av ett inducerbart morfologiskt försvar hos grodyngel. Man har länge vetat att yngel av olika grodarter har förmåga att plastiskt förändra stjärtfenan och kroppsformen vilket gör att sannolikheten för att undkomma en rovdjursattack ökar. Men hur detta regleras fysiologiskt har varit okänt fram till för några år sedan då en nordamerikansk forskargrupp visade att regleringen styrs av stress. Till att börja med visade de att kortikosteron-nivåerna hade ett positivt samband med predatortätheter i det vilda. Men än mer intressant var att man genom att tillsätta kortikosteron i vattnet kunde inducera de morfologiska försvar hos grodyngel som annars predator-exponering inducerar. Som pricken över i:et visade de att man kunde blockera uttrycket av de morfologiska försvaren hos predator-exponerade grodyngel genom att tillsätta en substans, metyrapon som bromsar produktionen av stresshormoner. Metyrapon används som läkemedel för att lindra effekterna av Cushings syndrom, d.v.s. överproduktion av kortisol. I artikel II tillämpar jag i princip samma experimentdesign men använder kortisol istället för kortikosteron och kroppsimplantat istället för fri exponering i vattnet. Som nämnts ovan har jag funnit att predator-exponering, förutom att göra rudans kroppsform betydligt högre dessutom inducerar en betydligt mörkare kroppsfärg. Så vad hände med rudorna som fick kortisol? Jo, i rak motsats till min initiala prediktion som baserades på de tidigare försöken på grodyngel, gjorde kortisol rudorna ljusare i kroppsfärgen och lägre i kroppsformen än kontrollfiskar som levt i

fred och frihet (i labmiljö) utan fiender. För att förklara dessa resultat på ett rimligt vis krävs först en introduktion till ryggradsdjurens stressaxel.

Stressaxeln (Hypotalamus-Hypofys-Adrenal-/Interrenal-axeln) är för oss människor precis som för alla andra ryggradsdjur ett livsviktigt system, även om det på senare tid har tagit död på en del av oss på grund av systemets missanpassning gentemot vårt moderna samhälle. Nåväl, via yttre stimuli, exempelvis åsynen av en naturlig predator, aktiveras detta hormonella feedbackmaskineri via signaler från hjärnan och sympatiska nervsystemet. Responsen sker omgående och har till syfte att förbereda individen fysiologiskt för den akuta faran; ”fly eller fäkta”. Inom loppet av några sekunder har förhöjda nivåer av så kallade katekolaminer (adrenalin och noradrenalin) utsöndrats i individens blodomlopp. Ett par minuter senare, som svar på en uppreglerad POMC-gen leder ökade halter av ACTH till ökad produktion och utsöndring av stresshormoner från binjurarna, så kallade glukokortikoider (kortisol/kortikosteron). När stresshormonerna, vilka är slutprodukten, ökar i koncentration skickas en signal åter till hjärnan om att bromsa systemet, detta för att inte ordagrant stressa ihjäl oss. Det är detta negativa feedbacksystem som är den troliga förklaringen till varför jag fann motstridiga resultat i paper II. En direkt ökad kortisolkoncentration utan ett fysiologiskt stresspåslag via sinnesintryck bör per automatik generera en nedreglering av stressaxelns processer. En nedreglering som då ska leda till minskade mängder av melanin-stimulerande peptider från POMC, vilket i sin tur kan förklara varför rudorna blev bleka (och kanske också varför de antog en relativt låg kroppsform). På samma sätt kan ett ökat uttryck av POMC från stressen av en närvarande predator förklara varför rudor blir mörka (och kanske också varför de antog en relativt hög kroppsform).

Ett annat fysiologiskt system som visat sig ha en nyckelfunktion i uttrycket av fenotypiska karaktärer är serotoninsystemet. Detta system har bland annat kunnat kopplas till individuella skillnader i proaktiva versus reaktiva beteenden d.v.s. individens riskbenägenhet, aggressivitet och aktivitetsgrad. Dessutom interagerar serotonin-systemet med stress-systemet, om något så är denna interaktion komplex, men kortfattat kan man säga att serotonin leder till reducerad stresskänslighet. Intressant nog så har tidigare studier också visat att serotonin har stor effekt på sociala beteenden. Ett klassiskt exempel är länken mellan individuella skillnader i hjärnans serotoninnivåer och social status hos markattor. Alfa-hanen är den som har högst serotoninnivåer men de sjunker snabbt så snart dominansen går förlorad. Än häftigare är att man kunnat visa att den här hierarkin är flexibel och går att manipulera med ganska enkla knep, som att medicinera hanar med relativt låg social status med selektiva återupptagningshämmare (SSRI). De medicinerade hanarna ändrar då sitt beteende vilket generar en högre status hos flockens honor något som indirekt sprider sig till gruppens hanar. Då mina handledare tidigare visat att rudor med olika personligheter

investerar olika mycket i det morfologiska försvaret så testade jag i paper III effekten av SSRI (fluoxetin) på både beteende och plasticitet i det morfologiska försvaret (kroppshöjd). Vidare skulle en minskad stresskänslighet ha potential att dämpa uttrycket av det morfologiska försvaret, eftersom jag i paper II och paper IV ger stöd åt hypotesen för ökad stress hos predator-exponerade rudor. Dessutom har SSRI kommit att bli en tämligen vanligt förekommande medicin mot psykisk ohälsa vilket gjort att detekterbara nivåer numer förekommer i olika vattendrag. Då serotoninsystemets receptorer är evolutionärt konserverade så finns det en överhängande risk att vårt användande av dessa substanser kan komma att påverka andra arters fysiologi. Detta diskuteras mer i detalj i paper III i samband med mina resultat från detta experiment men kortfattat kan nämnas att jag fann en effekt av serotonin på rudors beteende. På kort sikt hade en hög dos av fluoxetin en tydlig effekt på rudornas beteende, de uppvisade en mer försiktig, feg attityd i förhållande till de artfränder som exponerades för en låg dos alternativt ingen dos alls. Den observerade beteendeförändringen försvann dock efter längre tid vilket förmodligen tyder på att de anpassat sig. Detta påminner en aning om behandling med SSRI hos deprimerade människor där det inte är ovanligt att SSRI-behandling kan göra patienter initialt sämre med suicidalt beteende som en allvarlig biverkning. Kanske än mer intressant var att vi fann stor effekt av fluoxetin på rudornas kroppsform. Men medan gädd-exponering inducerade en högre kroppsform genom att öka ryggens höjd så ledde fluoxetin till en ökad kroppshöjd via en större mage, en dorsal kontra ventral tillväxt, med andra ord diametralt motsatta effekter. Jag förväntade mig att finna en negativ effekt på kroppshöjden av fluoxetin eftersom jag predikterade att administration av en SSRI skulle reducera stresskänsligheten och då medföra en dämpad morfologisk respons mot predationsrisk. Men något stöd för detta fann jag alltså inte, däremot fann jag starkt stöd för stora könseffekter i paper IV så låt mig berätta om det istället.

Individuell variation är i naturen en regel och inget undantag, en regel som absolut kan tillämpas på rudornas uttryck av deras morfologiska försvar mot rovfisk. Under mina initiala experiment såg jag ofta hur vissa individer snabbt reagerade på en närvarande predator och byggde om sin kropp med tämligen dramatiskt slutresultat. Andra individer, däremot, var mer eller mindre likformiga i slutet som i början av experimenten. Detta fick mig givetvis att fundera på den bakomliggande orsaken till denna variation i plastisk kapacitet. I synnerhet eftersom variationen i sig skulle kunna ge direkta ledtrådar till det underliggande maskineri som reglerar rudans plastiska kroppsform. Nåväl, eftersom jag är övertygad om att biologiska könsskillnader existerar (sorry Cordelia Fine) ja jag vill till och med påstå att könseffekter är tämligen vanliga (sorry igen Cordelia Fine), så var effekten av kön en självklar första hypotes att testa. Generellt sett så investerar honor mer i avkomman än hanar. Hos människan visar

produktionen av antalet ägg kontra produktionen av antalet spermier över en livstid en tydlig indikation på att äggen står i högre kurs. Lägg därtill en nio månader lång graviditet i kombination med en förlossningssmärta som saknar motstycke så blir skillnaden i den reproduktiva insatsen mellan män och kvinnor väldigt tydlig. Den här orättvisan i reproduktiv investering görs inte bara gällande för oss människor utan är relativt universell i djurriket. Att studera könseffekter är dessutom av ytterligare intresse ur det stress-hypotesdrivna perspektiv som jag arbetat utifrån. Flertalet moderna studier har nämligen visat att det ofta föreligger stora skillnader i hur honor och hanar reagerar på stress. Vissa forskare menar till och med att förberedelsen för "fight or flight" som ofta beskriver den adaptiva funktionen av stress är typiskt för hanar och att honor av många arter istället utvecklat en stressrespons som i högre grad gynnar avkomman, en så kallad "tend-and-befriend" reaktion. Med dessa argument initierade jag en kombinerad fält- och laboratorie-studie på rudor för att utforska potentiella könsskillnader i morfologiskt försvar mot predation. Enligt prediktion fann jag att vildfångade hanar från sjöar med hög predationsrisk uttrycker mer kroppsligt försvar än honor från samma populationer. Vidare kunde jag visa att predator-naiva hanar inducerar mer morfologiskt försvar än vad predator-naiva honor gör under kontrollerade laboratorieförhållanden. Jag föreslår att denna könseffekt ligger till grund för en avvägning där honor inte har råd med samma investeringar som hanar då deras reproduktiva investering är högre. I ett led att försöka öka vår förståelse kring den underliggande fysiologin så samlade jag in hjärnor från honor och hanar som antingen levt med eller utan gädda under sex månader, d.v.s. samma fiskar som visade denna könseffekt. Från hjärnorna extraherades RNA för att möjliggöra genuttrycksanalys på den högintressanta POMC-genen. Jag fann att rudor har tre kopior av denna gen men än mer intressant så fann jag att predator-exponerade hanar skiljer sig i uttrycket från övriga grupper genom att en av dessa POMC-kopior var uppreglerad inom denna grupp. Detta kan indikera att hanar reagerar annorlunda på predator-inducerad stress jämfört med honor och att denna skillnad i stress-respons skulle kunna ligga till grund för den observerade könseffekten i morfologiskt försvar. Könsskillnader är visserligen ett modernt samtalsämne men så här mitt i rådande pandemi-tider är kanske nästa stycke än mer aktuellt.

Eftersom stress i kronisk form kan medföra negativa effekter på diverse funktioner har jag i paper I undersökt om predationsrisk förändrar immunförsvaret hos ruda. Enligt klassisk livshistorieteori har vi alla begränsade resurser. Vi måste därför väga varje investering gentemot en annan. Med denna grund undersökte jag om det finns en avvägning mellan olika försvar. Mer exakt så undersökte jag om rudor som uttrycker en hög magnitud av morfologiskt försvar mot predation får försämrat internt försvar mot patogener d.v.s. om investering i att skydda sig mot en naturlig fiende försämrar

försvaret mot andra fiender? Intressant nog fann jag att viktiga variabler i immunförsvaret förändras av predationsrisk och jag fann också ett starkt samband mellan de båda försvaren. Mina resultat ger dock inte stöd åt en avvägning mellan morfologisk och fysiologisk investering, istället föreslår jag att mina resultat talar för stora individuella skillnader i hur väl individer hanterar predationsrisk. Med detta nämnt har jag nog lyckats sammanfatta den fysiologiska delen av mitt doktorandprojekt och kommer således avsluta med en kort beskrivning av övriga saker jag fokuserat på.

Utöver dramatiska förändringar i kroppsform så presenterar jag här nya data som indikerar att rudan har utvecklat plastisk kapacitet i en rad andra karaktärsdrag. Som redan nämnts så visar jag i paper II att predator-exponering inducerar en mörkare kroppsfärg hos rudor. En mörkare kroppsfärg skulle kunna vara ett fördelaktigt skydd mot predation. Dels då en mörkare färg kan tänkas öka kontrasten av individens siluett som i kombination med en högre kroppsform kan skicka signaler till predatorn om att en attack är onödig, "jag är ändå för stor för ditt gap". Vidare skulle en mörkare kroppsfärg kunna vara adaptiv i kombination med övergången till en nattaktiv livsstil som jag visar i paper V. Generellt kan man säga att predationsrisk reducerar nivån på rudornas aktivitet d.v.s. de simmar kortare sträckor per dag då de misstänker att en predator ligger och lurar i närheten. I paper V visar jag dessutom att de skiftar dygnsrytm och blir mer nattaktiva när de samexisterar med gädda. En sådan plastisk förändring i dygnsrytm kan vara direkt fördelaktig då gäddor som är relativt visuellt orienterade jägare är mer aktiva under dygnets ljusare timmar. Men vad som kanske var än mer spännande var att jag fann bevis för att rudor också förändrar sina ögon när de utsätts för predationsrisk. Mer exakt fann jag att de förstorar sin pupill, en förändring som jag med hjälp av en matematisk modell kunde illustrera vara fördelaktig, i synnerhet för att se små objekt under låga ljusförhållanden. Denna plastiska pupillförstoring kanske inte låter så märkvärdig för oss människor då våra ögon har utvecklat muskler som kan reglera pupillens storlek som en direkt respons på förändringar av inkommande ljus. Ett sådant muskelsystem saknas dock hos de flesta fiskar och hur rudan lyckas förstora sin pupill trots detta är en fråga för framtida studier. Vad man däremot kan misstänka är att en större pupill är fördelaktig när det kommer till att finna mat och upptäcka predatorer, inte minst när man är aktiv under natten då ljusförhållandena är som sämst.

Så, för att göra en lång historia kort: Jag har spenderat otaliga dagar med rudor i olika färger och former. Jag har studerat dem i fält och i labb. Jag har injicerat dem med ett ämne från sjukdomsorsakande kolibakterier. Jag har opererat in elektroniska chip i deras magar. Jag har gett dem implantat med olika substanser. Jag har hällt lyckopiller i flytande form i deras vatten. Jag har tagit blod- och organprover. Jag har undersökt deras kön. Jag har tagit fler bilder på rudor än på mina egna barn. Jag har smygfilmat

deras nattliv, rudornas, inte barnens. Jag har haft roligt. Jag har lärt mig massor och med det fått inse att jag inte alls kan så mycket. Jag har fler frågor nu än när projektet startade och hoppas därför framtiden erbjuder mig mer kvalitetstid med dessa transformers.

Introduction

This is a thesis about phenotypic plasticity in morphological anti-predator traits and the physiological control of their expression. It is about stress physiology and hidden costs of co-existing with predators when being a phenotypically plastic prey. Lastly, it is about sex, drugs and a suite of plastic defence strategies.

Keywords: Inducible defences, Phenotypic plasticity, Stress physiology, Cortisol, Immune function, Crucian carp, Predator-prey interactions, Anti-predator defences, Trade-offs, Sexual dimorphism, POMC, Pro-opiomelanocortin, Visual ecology, Eye evolution, Colour change, Melanin, Eco-immunology, Pace-of-life

Predator-prey interactions

Organisms are constantly exposed to numerous and disparate factors that ultimately influence their fitness through effects on survival and reproduction (Darwin 1859). To maximize lifetime fitness in such complex and mercurial environments, individuals must balance competing demands according to the current strength of each selective agent. Here, predation is without any doubt one of the major forces in the natural selection of prey phenotypes and it has played a ubiquitous and significant role for the divergence and complexity of organisms across the natural world. The act of predation is probably as old as life itself (Bengtson 2002), and when defined as the conflict where one organism is killing another organism for energetic demands, it involves the vast majority of lifeforms on our planet (Abrams 2000). As the failure to avoid predation is definitive, i.e. death, evolution has produced a plethora of antipredator traits, ranging from physiology and morphology to specific behaviours (e.g. Cott 1940; Brodie Jr 1977; Ydenberg & Dill 1986; Brönmark & Miner 1992; Young, Brodie Jr & Brodie III 2004; Price, Friedman & Wainwright 2015; Hodge *et al.* 2018). Due to its importance, predator-prey interactions have for long been a central pillar in evolutionary biology and ecology and are in many ways still a vibrant research area. On top of shaping prey phenotypes, predation has a major role in the construction of the

natural world. Direct lethal effects of predation have obvious and strong effects on whole ecosystems, from regulating prey abundances to shaping the structure of entire communities (Sih et al. 1985, Kerfoot and Sih 1987). Further, predators may, by their mere presence in a habitat, cause significant indirect effects, e.g. by altering physiological, behavioural and demographic traits in prey. Such effects have become a research field in its own right and is nowadays often referred to as the "ecology of fear" (Brown, Laundré & Gurung 1999; Gaynor *et al.* 2019). However, there is an ongoing debate whether "fear" is the correct word to define predator risk effects, basically because we have no understanding of the cognitive and emotional properties of perceived predation risk: "*Females of many species avoid mates of low quality, but do they fear them? Vegetarians avoid meat, but do they fear it?*"" – Creel *et al* (2009). Yet, regardless a sense of fear or not, predation risk often invokes a stress response in prey as is formulated in the *predation stress hypothesis* (Romero 2004), which I further discuss in **paper I-IV**. Predators may also constrain movement, activity and feeding in prey, resulting in reduced foraging efficiency and less energy available to allocate into reproduction, growth and survival, a mechanism that has been termed the *predator-sensitive food hypothesis* (Metcalfe, Huntingford & Thorpe 1987; Sinclair & Arcese 1995; Paszkowski *et al.* 1996; Romero 2004; Creel *et al.* 2007; Zanette *et al.* 2011). Recently, it has been proposed that prey can assess predation risk and respond either proactively or reactively to maximize survival chances, i.e. the *control of risk hypothesis* (Creel 2018). Here, proactive responses, such as migration to areas with lower risk or shoaling behaviour resulting in individual risk dilution, are expected to occur in stable and predictable environments and, thus, result in food-mediated costs. In contrast, reactive responses such as to fight or flight or demonstrate aposematic signals are more likely to occur in varying and unpredictable environments, and then result in stress-mediated costs (Creel 2018). In sum, non-lethal effects originating from the mere presence of predators may ultimately cascade like an echo of consequences through the whole ecosystem (Schmitz, Beckerman & O'Brien 1997; Hawlena & Schmitz 2010), and may even be a more potent driver of prey population dynamics than direct lethal effects (Preisser, Bolnick & Benard 2005).

The rabbit runs faster than the fox, because the rabbit is running for his life while the fox is only running for his dinner – Dawkins (1976). [Photo: Manfred Isberg]

Predation risk is defined as an individual's likelihood of (i) encountering a predator and (ii) being killed if encountered (Lima & Dill 1990). From a predator's perspective, a sequence of events has to be executed successfully to gain the real trophy of hunting, i.e. energy. This foraging cycle of predators involves to: search, encounter, detect, attack, capture, ingest and digest the prey (Holling 1965; Lima & Dill 1990; Brönmark & Hansson 2012). Therefore, to disrupt this foraging cycle and secure future existence prey organisms should evolve adaptations that result in reduced efficiency during one or several steps in the process and preferably as early as possible in the cycle (Endler 1991). Anti-predator strategies are therefore not only prevalent in the natural world, they are also multifaceted due to the many alternative defence strategies. For example, prey may evolve primary defences that serve to avoid detection via e.g. refuge use (Wojdak 2009), migration (Hulthén *et al.* 2015) or camouflage (Cott 1940), or by advertising themselves as unprofitable to predators via e.g. mimicry or warning signals (Cott 1940; Endler 1978). If detected, then secondary defences such as fleeing (Ydenberg & Dill 1986), freezing (Höglund *et al.* 2005) or to use toxic chemicals (Brodie Jr 1977; Bakus 1981) or a defensive morphology (Brönmark & Miner 1992;

Young, Brodie Jr & Brodie III 2004; Hodge *et al.* 2018) can increase the chance of surviving the prey-capturing process. In this thesis, I have mainly focused on the latter, i.e. morphological anti-predator traits. Such defence strategies may evolve over generations and become constitutively expressed in the phenotype irrespectively of the current risk situation, as for example body armour in the nine-spined stickleback (Välimaki, Herczeg & Merila 2012), the modified stiff and spiny hairs of hedgehogs (Brodie Jr 1977) or the bony horns in horned lizards (Young, Brodie Jr & Brodie III 2004). However, during the last decades it has become increasingly evident that some prey organisms also respond to predation risk by phenotypic plasticity in key defence traits, i.e. they have the ability to tune various aspects of the phenotype to the prevailing risk of predation (Tollrian & Harvell 1999).

Phenotypic plasticity

Adaptive phenotypic plasticity can be defined as the ability of an organism to express changes within the phenotype that enhance fitness in the current environment (Agrawal 2001; Pigliucci 2001; West-Eberhard 2003). Stem cells are a great parallel for illustration, they all share identical genotypes but develop into unique cell types via specific environmental stimuli (Goldberg, Allis & Bernstein 2007).

Some history

It is now more than 100 years since the German zoologist Richard Woltereck carried out the first experiment related to organismal plasticity (Woltereck 1909). At that time, Mendel's work on heredity had been rediscovered, and adhered a saltationistic view, i.e. that the evolution of species occurs by large mutational changes passed from one generation to the next (Falk 1995; Pontarotti 2009). This view of saltationism is of course in direct contrast to Darwin's idea of evolutionary processes as a gradual and continuous change under natural selection (Darwin 1859). However, Woltereck, who was a Darwinian defender, set out to falsify the idea of saltationism by rearing *Daphnia* in different nutrient levels and observing any changes in phenotypic traits related to the different treatments. *Daphnia* species have short generation times and can reproduce asexually (Stollewerk 2010), so Woltereck elegantly used individuals originating from single lines, i.e. individuals having identical genotypes. During the course of his experiments, he noticed a continuous phenotypic change among individuals reared in different nutrient levels. For example, head size differed between treatments, a function he illustrated in a graph showing a phenotypic curve with nutrient levels on the X-axis

and relative head-height on the Y-axis. He called those curves for "reaction norms" or "norms of reaction"(see Figure 1), and argued that it is the norms of an individual's reaction to the environment that are inherited in gradual steps to future generations, a conclusion that falsified the idea of saltation and single-step speciation (Heams *et al.* 2014; Sommer *et al.* 2017). At around the same time, the Danish botanist and geneticist Wilhelm Johannsen coined the concepts of *genes, genotype* and *phenotype*, and discussed the interactions between them (Johannsen 1911).

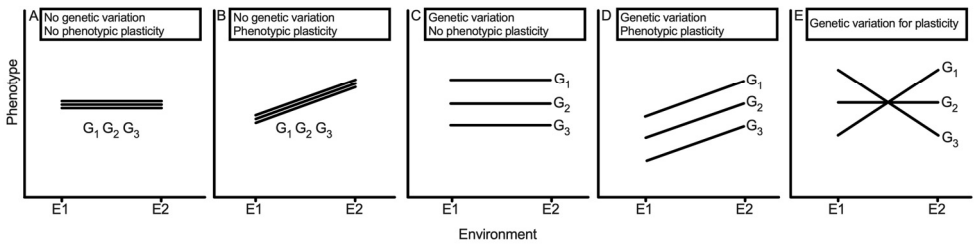


Figure 1. A theoretical representation of reaction norms for three unique genotypes (G_1 – G_3) originating from five different populations (A-E). Each population represents different properties of genetic variation and phenotypic plasticity. Figure modified from (Hegg 2020).

Three decades later, the British scientist Conrad Waddington broke ground with ideas of *genetic assimilation* and the *epigenotype* (Lees & Waddington 1942; Waddington 1942; Waddington 1956; Waddington 1959). Waddington, who worked with *Drosophila,* performed various experiments and showed that he could environmentally induce a character in the phenotype that subsequently was inherited by the following generations despite the lack of environmental stimuli for the current trait expression, i.e. the trait was fixed in the population via *genetic assimilation*. Later, Waddington coined the framework of an epigenetic landscape that he defined as the complex processes of development that eventually result in the adult characteristics of organisms, see Figure 2 (Waddington 1942). Hence, Waddington realised that environmental cues can alter development of traits and that such changes can be inherited to subsequent generations. Today, epigenetics has become its own flourishing field of research focusing on any heritable change in gene or phenotypic expression that are caused without any changes in Watson-Crick base-pairing of DNA (Goldberg, Allis & Bernstein 2007), for example transgenerational plasticity in anti-predator strategies (Richter-Boix, Orizaola & Laurila 2014; Bell, McGhee & Stein 2016).

The first major conceptual advances within the research field of phenotypic plasticity was laid by Anthony Bradshaw in 1965, when he argued that plasticity must be genetically regulated (Bradshaw 1965). This important part of the theoretical underpinnings of phenotypic plasticity has, however, been discussed heavily just until

recently, where plasticity and genetics was argued to be contrasting processes, whereas today the general consensus is that plasticity without any doubt should be viewed as a property of the genotype (Pigliucci 2005). Moreover, Bradshaw argued that the control of a phenotype ranges between a (i) rigorous canalisation into specific pathways, i.e. stability without plasticity, to (ii) canalization into contrasting pathways resulting in a possibility of two distinctly divergent and determined forms, or, (iii) the control can be broad with continuous plasticity. However, here he came with an important argument by stating that this control is not to be viewed as a general property of the whole genotype, instead it is a control of specific traits influenced by specific environmental cues. From his studies on plants, that in contrast to the animal kingdom basically consist of stationary organisms, Bradshaw successfully proposed the conditions that would favour the evolution of adaptive phenotypic plasticity, see next section (Bradshaw 1965). Since then, interest in phenotypic plasticity has grown immensely (see Figure 3), resulting in a solid framework for the theoretical underpinnings (Pigliucci 2001; West-Eberhard 2003; Pigliucci 2005) and an understanding of its role for producing novel phenotypes and even species (Pfennig *et al.* 2010; Levis, Isdaner & Pfennig 2018).

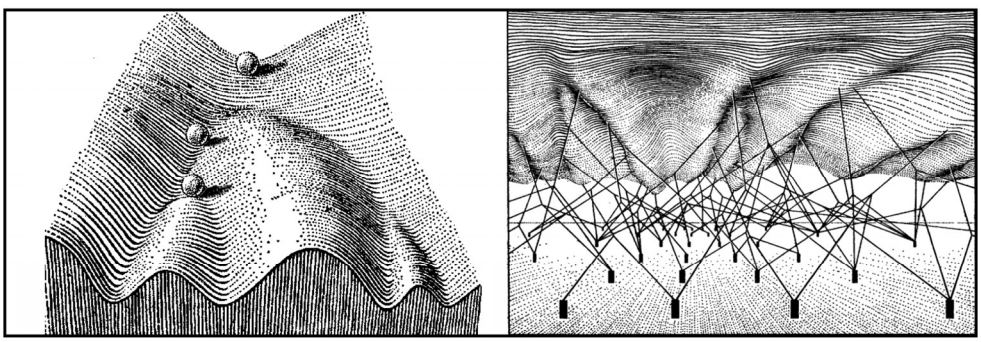


Figure 2. The rolling ball in (A) Conrad Waddington's **"**epigenetic landscape" represents the process of cell (or trait) development. The ball may, depending on possible trajectories, result in a final outcome different to another outcome following another pathway. To the right, Waddingtons illustration of how the epigenetic landscape looks from behind where the pegs are genes and the strings are the products from the different genes i.e. proteins. Modified from Waddington (1957).

Plasticity in anti-predator traits

From initially being described as an evolutionary strategy among plants (Haukioja 1980) and asexual invertebrates (Gilbert 1966), inducible morphological defences are nowadays a well-described strategy to cope with predation across a diverse range of taxa (Tollrian & Harvell 1999). Spine length and helmets in different species of water fleas (Dodson 1988; Tollrian 1995) and shell shape and colouration in freshwater snails

(Brönmark, Lakowitz & Hollander 2011; Ahlgren *et al.* 2013) are well-studied traits showing plasticity in response to changes in predation risk. Even vertebrate species respond to changes in predation risk with remarkable morphological adaptive plasticity. Here, aquatic and semiaquatic organisms are the most represented organisms in the literature. For example, amphibian tadpoles are known to go through rapid changes in body shape, body colouration and tail size when exposed to chemical cues from natural predators (McCollum & Leimberger 1997). Moreover, some fish species can alter external characters such as the size of fins, eyes and body depth to reduce the risk of predation from piscivorous fish (Brönmark & Miner 1992; Eklöv & Jonsson 2007; Ab Ghani, Herczeg & Merilä 2016; Svanbäck & Johansson 2019). Further, as interest in predator-induced plasticity increases it is predicted that more and more hidden and cryptic defence adaptations will be recognized. To follow the statement in the nowadays classic book **The Ecology and Evolution of Inducible Defences**: "*The obvious cases in well-studied systems have been found first; in animals where the inducible traits are visible and must only be linked to inducing agents. However, as other systems are better studied, more cryptic defences become visible. An induced helmet is clearly easier to detect than a change in reproductive effort in the same organism*" – Tollrian and Harvell (1999). Hence, inducible defences against predation is a classic example of phenotypic plasticity, and numerous examples of such conditional strategies against predation have now been described across a diverse range of taxa

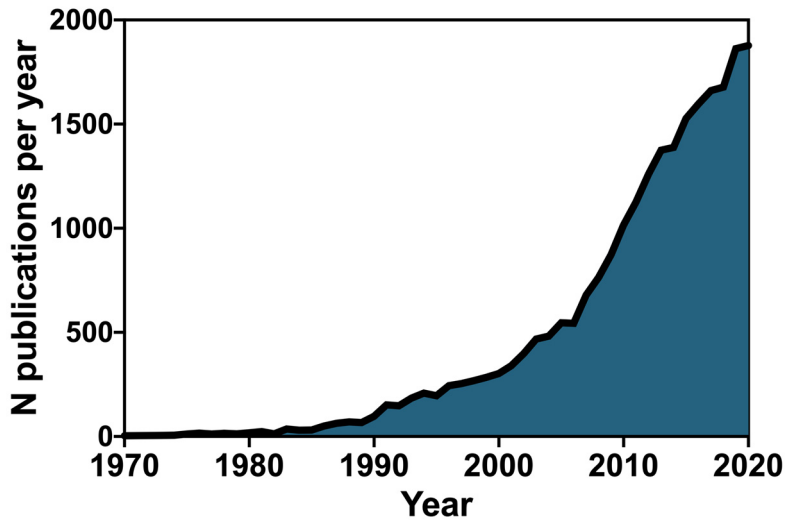


Figure 3. Annual data for the number of research articles published within the field of "phenotypic plasticity". The data was obtained from Web of Science including all databases, using the search word "Phenotypic plasticity" as topic, ranging from year 1970 to 2020.

During the last decades, a great deal of research has been devoted to shed new light on the question when evolution will favour an inducible over a constitutive defence (Harvell & Tollrian 1999; Tollrian & Harvell 1999). **First**, prey species must be able to detect and respond to reliable cues that communicate the level of risk. **Second**, the induced defence must serve its purpose, i.e. reduce the risk of becoming a prey. **Third**, predation risk must be spatially or temporally variable, and, **fourth**, the induced defence must carry costs (Tollrian & Harvell 1999). The first point relates to the importance of being able to detect and respond to environmental heterogeneity, via e.g. the presence or absence of chemical cues from natural predators (Brönmark & Hansson 2012; Mitchell, Bairos-Novak & Ferrari 2017). However, the two last points separate the different evolutionary pathways between inducible defences and constitutive defences. If predation risk varies and the defence expression is associated with costs (e.g. Innes-Gold, Zuczek & Touchon 2019), fine-tuning of the expression of defences to the current risk level should result in a closer phenotype to environment matching, and, accordingly, phenotypically plastic prey should enjoy fitness benefits as compared to prey with constitutive defences (Brönmark & Miner 1992; McCollum & Van Buskirk 1996). However, from the perspective of phenotypes it is clear that the risk of becoming a prey is seldom equal, not even for individuals of the same population.

Individual variation

Nowadays, we know that individuals of the very same population can differ substantially in the expression of inducible traits (Hulthén *et al.* 2014; Nagano & Doi 2020). For example, we know that the capacity for plasticity might differ during ontogeny, where different phenotypic directions are defined under specific developmental windows (Hochberg *et al.* 2011). For instance, Schneider *et al.* (2014) found that diet-induced plasticity in jaw morphology of a cichlid fish was initiated after three months of treatment exposure, and that the time period between three and five months of environmental exposure was a critical period due to major transcriptional changes in the regulatory gene network controlling jaw development. Hence, developmental windows of varying programmability may explain some of the individual variation that we observe. Moreover, intraspecific variation in phenotypic plasticity may differ between sexes, for example, males, but not females, of a western African river cichlid show phenotypic plasticity in numerous morphological anti-predator traits (body depth, dorsal spines, eye size; (Meuthen *et al.* 2018). Variation in inducibility can also reflect underlaying differences in animal personalities, where for example individual variation in risk taking behaviour (boldness) has been linked to the degree of plastic expression of a morphological defence (Hulthén *et al.* 2014). Studies on animal personality has grown immensely in popularity during the last years (Bell

2012; Furtbauer *et al.* 2015), resulting in a universal terminology and a new theoretical framework. In brief, personality in animals can be defined as repeatable inter-individual differences in specific behavioural traits, for example individual position on the boldness continuum. However, when a suite of behavioural characteristics are stable over time and co-vary across different contexts we call it a *behavioural syndrome* (Sih, Bell & Johnson 2004). Such behavioural variation further produce varying *stress coping styles* which directly relates to the reactive *versus* proactive anti-predator responses as I discussed earlier (see under Predator-prey interactions). Variation in stress coping styles become intriguing in the light of the causal physiology (Koolhaas *et al.* 2010). Here, it is mainly two physiological systems that have received particular attention in recent years: the melanocortin system and the vertebrate stress axis. I should here mention in brief that melanocortins are a small group of peptide hormones mainly originating from one single gene, the POMC (pro-opoimelanocortin) gene, see **paper IV**. Importantly, POMC-derived melanocortins have, except from being involved in melanin-based colouration, a major role within the vertebrate stress axis. Here, due to its fundamental role as the proximate agent for the production and release of glucocorticoids, ACTH (adrenocorticotropic hormone) should be mentioned. This multifaceted effects of POMC-derived peptides creates an intriguing covariance between melanin-based colouration and stress coping styles. In salmonids, for example, the presence of dominant individuals is known to induce a darker body coloration in more subordinate conspecifics. Proximately, the change in body darkness are caused by higher concentrations of POMC-derived melanocortins that stem from the social stress of being a subordinate (Höglund, Balm & Winberg 2000). Additional studies on teleost species have linked a high number of melanin-spots or being darker in general to a proactive behaviour whereas a low number of melanin-spots or having a paler colouration in general have been linked to a reactive behaviour (Kittilsen *et al.* 2009; Schweitzer, Motreuil & Dechaume-Moncharmont 2015). Furthermore, Ducrest, Keller and Roulin (2008) hypothesized that individual differences in melanin-based colouration among different species of mammals, birds, amphibian and fish could be used to predict behavioural syndromes, as the gene (POMC) involved in melanin-based colouration are further known to regulate the stress-axis and therefore also behavioural characteristics. Indeed, vertebrates with darker body colouration were found to be more aggressive, have higher sexual activity and were more resistant to external stressors than individuals with paler body colouration (Ducrest, Keller & Roulin 2008). In **paper II,** I demonstrate predator-induced body darkness in a prey species as a result of increased melanogenesis from predation risk *per se*. Hence, this interaction between the melanocortin system, behavioral characteristics and stress physiology creates an intriguing link to the regulatory machinery behind plasticity in anti-predator traits.

Predator-induced stress

Stress has been defined as a nonspecific response of the body to any demand made upon it (Selye 1973). This definition is very broad and includes responses such as shivering when being cold and sweating when being warm, etc. Therefore, for the purpose of this thesis, I will narrow the definition of stress to only refer to the specific physiological response that prey show when they in some way perceives a risk of predation. When free from external stressors, as most animals are most of the time, the steroid hormones named glucocorticoids (stress hormones) act as key regulators of numerous essential daily life processes. For example, varying basal levels of glucocorticoids have been shown to influence diel rhythm, energy balance, the reproductive system, foraging behaviour and locomotor activity (Landys, Ramenofsky & Wingfield 2006). However, the *predation stress hypothesis* suggests that predator exposure causes elevation of glucocorticoids in prey. Such increase in glucocorticoids arise from direct modification of the vertebrate stress axis, see Figure 4 (Sapolsky, Romero & Munck 2000; Clinchy, Sheriff & Zanette 2013; Oliveira *et al.* 2014; Hammerschlag *et al.* 2017). The stress response, in brief, has evolved to be self-regulated via a negative feedback system where the majority of steroid actions, such as glucocorticoid production and secretion, are under genetic control from the POMC gene in the pituitary gland. Synthesis of POMC produces a precursor (pro-opiomelanocortin) resulting in multiple peptide hormones with various phenotypic effects (Harris, Dijkstra & Hofmann 2014; Navarro *et al.* 2016). Of particular interest here are the so called POMC-derived melanocortin peptides: (i) ACTH and (ii) α-melanophore-stimulating hormone (α-MSH). As mentioned above, earlier studies have demonstrated their principal roles in the process of melanogenesis and pigment dispersion/aggregation (Fujii 2000; Ducrest, Keller & Roulin 2008; Leclercq, Taylor & Migaud 2010; Cerda-Reverter et al. 2011; Sköld et al. 2015; Cal et al. 2017), but they are also main regulatory agents in the vertebrate stress axis (Aguilera 1994; Sapolsky, Romero & Munck 2000). For example, enhanced glucocorticoid concentrations from production and secretion of melanocortins subsequently suppresses the pituitary expression of POMC, i.e. reduces levels of ACTH and α-MSH (Drouin *et al.* 1989; Aguilera 1994; Slominski *et al.* 2000). This self-regulation of the stress axis is important in order to retain homeostasis after exposure to an acute stressor and to avoid lethal effects from high glucocorticoid levels.

Let's take a break here with a small parenthesis, and bridge these processes as recently discussed with a rare endocrine disorder named Addison's disease. This disorder arise from problems with the production of cortisol in the adrenal gland, resulting in pathologically low levels of cortisol. Such low levels of cortisol result in a lack of negative feedback control of the stress axis in affected individuals. This lack of negative feedback

will stimulate a continuous release of ACTH from upregulation of POMC, leading to hyperpigmentation as one of the major symptoms (Allen & Sharma 2020). This is intriguing in the light of trait correlations from pleiotropy in the melanocortin system, remember the discussion above under "Individual variation".

Let's move on. The adaptive value of the acute stress response lies in preparing the individual for a dramatic expenditure of energy followed by homeostatic recovery (Sapolsky 2004). Moreover, the action of secreted hormones occurring within seconds (catecholamines) to minutes (glucocorticoids) in response to a stressor results in resource mobilization along with enhanced cardiovascular tone and an increased flow of energy to exercising muscles which improves the chances to either fight or flight the dangerous situation (Sapolsky, Romero & Munck 2000). In addition, immune function parameters become upregulated, and specific cognitive capacities sharpened, whereas all aspects of reproductive physiology and feeding behaviour are inhibited (Sapolsky, Romero & Munck 2000). Several studies have investigated the proximate and ultimate functions of the stress response in prey organisms (Sapolsky, Romero & Munck 2000; Giesing *et al.* 2011; Oliveira *et al.* 2014; Hammerschlag *et al.* 2017). Therefore, it is nowadays well established that the release of glucocorticoids may result in pleiotropic effects on the phenotype, and, further, that the sensitivity and responsiveness of the stress axis often show a high degree of inter- and intraspecific variability (Sapolsky 1982; Sapolsky 1990; Höglund, Balm & Winberg 2000). Such variation can, at least partly, be explained by differences in local selection regimes. Here, predation risk seems to be an important driver for the evolutionary pathways of stress responses, where, for example, earlier studies have shown that prey organisms who often interact with their predators may evolve an attenuated response (e.g. Brown, Gardner & Braithwaite 2005). Such variation can be regional (Dahl *et al.* 2012), and be explained from differences in e.g. life history strategies (Boonstra 2013) between the sexes for example. Some researcher have even argued that the "fight-or-flight" response is particularly important in males since females of many species are significantly less aggressive and that evolution would never favour the option of flight in a mother having dependent offspring's (Taylor *et al.* 2000). Here, female responses to stress have been suggested to build on processes related to attachment and caregiving processes that ultimately would downregulate the HPA-axis. Hence, instead of a fight-or-flight response, females of many species may respond to stress by a "tend-and-befriend" response mediated by oxytocin and regulated by sex hormones and endocrine mechanisms (Sapolsky, Romero & Munck 2000; Taylor *et al.* 2000). However, as mentioned above, variation in stress responses is common and may be mediated by different evolutionary pathways caused by different selection regimes.

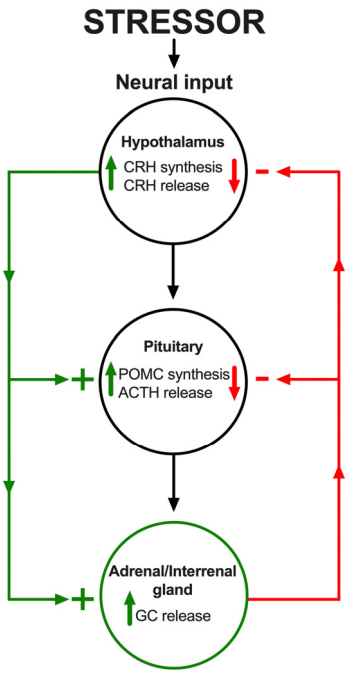


Figure 4. Outline of the vertebrate stress axis i.e. the HPA/HPI axis (Hypothalamus-Pituitary-Adrenal/Interrenal axis). A stressor is noticed and immediately followed by the release of CRH (corticotropin releasing hormone) by the Hypothalamus. CRH is transported to the anterior pituitary where POMC (pro-opiomelanocortin) is synthesised. POMC is subsequently cleaved into multiple peptide hormones, including ACTH (adrenocorticotropin hormone), which are released into the circulatory system, signalling to the adrenal gland to release GC (glucocorticoids) e.g. cortisol. In teleost fish and also amphibians, the release of cortisol occurs in specialised cells within the interrenal gland. Importantly, the stress response is self-regulated via a negative feedback system (red) where the production and secretion of GC are reduced from suppression of the POMC gene, caused by elevated GC concentrations.

Acute *versus* chronic stress

Stress as a biological concept has a long history of research, dating back to the American physiologist Walter B. Cannon who, a century ago, coined the terms *homeostasis* and the *fight-or-flight response*, respectively referring to the steady state of an organism's internal physiology, and the physiological processes causing an altered and adaptive behavioural response in animals when exposed to a stressor (Cannon 1915; Cannon 1932). The conceptual framework of homeostasis has nowadays been modified by involving the role of *allostasis (Sterling & Eyer 1988),* defined as the organism's ability to achieve stability through change. Hence, *allostasis*, in contrast to *homeostasis* reflects the internal processes of adjusting physiological parameters in a manner so that the phenotype is adapted to a new or changing environment (Ramsay & Woods 2014). With this in mind, it should be argued that all living animals, at least to some degree, show plastic capacity (Forsman 2015). However, some years after Cannons work, Hans

Selye (often referred to as the "Father of stress") recognized a clear difference in the response of an animal dependent on the duration of the stressor (Selye 1936). If short-term, as experienced by a zebra chased by a lion, the stress response will be short lived, i.e. homeostasis will be retained shortly after the stressor. However, if the stressor remains and become chronic, a set of symptoms will follow, and he named these the *general adaption syndrome* (Selye 1936). In essence, it includes three separate stages, starting with the (i) *alarm phase* i.e. when the stress axis is triggered by an external stressor and subsequently followed by (ii) a bodily *resistance* for the amplified secretion of stress-related hormones such as catecholamines and glucocorticoids. If the stressful event persists, a stage of (iii) *exhaustion* will follow where the stores of stress hormones are depleted, causing sickness and lethal effects for the organism (Selye 1946; Selye 1973). However, new research suggests that the major harmful effects from chronic stress are due to the energetic costs of having the response activated, that is, if you always defend yourself for one emergency you will trade-off mobilization of energy for other existentially important tasks, e.g. reproduction, energy storage, disease prevention, etc. (Gregory & Wood 1999; Cooke *et al.* 2003; Sapolsky 2004; Lankford *et al.* 2005). For example, an important negative aspect of chronic stress is the reduced capacity of immunity against infections, basically because it makes more sense for the body to allocate resources to fight or flight than to detect diseases that might kill you in years to come - when now is an emergency (Sapolsky 2004; Dhabhar 2014). Recently, it has been debated whether or not non-human animals experience chronic physiological stress in their natural environments (Sapolsky 2004; Boonstra 2013; Clinchy, Sheriff & Zanette 2013). Here, predator-induced stress has traditionally been considered as an acute and transitory response, a point illustrated in Robert Sapolsky's well-cited book *Why Zebras Don't Get Ulcers* (2004). Based on our knowledge regarding the maladaptive effects of chronically induced stress, Sapolsky argue that it should have been selected against and is therefore non-existent in nature. In contrast to this earlier view, where chronic stress was assumed to only carry maladaptive effects, it has become increasingly recognized that the effects are context dependent. For example, snowshoe hare have chronically high plasma cortisol concentrations during times when predation risk is high (Boonstra *et al.* 1998), and glucocorticoid concentrations in cape fur seals show high correlation with regional number of shark attacks (Hammerschlag *et al.* 2017). However, elk respond to variation in predation risk from wolfs via altered behaviour but not by altered stress physiology (Creel, Winnie & Christianson 2009). So, if chronic stress increases the risk of sickness and reduced fitness through energetic and reproductive costs, why do some species show signs of chronic elevation of physiological stress in the wild? In addition, a recently published review demonstrated that adjustment to captivity is highly species specific, where some animals rapidly adapt to captivity whereas other never do (Fischer & Romero 2019). The answer for this

interspecific variation in stress coping styles may lie in divergent adaptations to differences in lifespan and life history strategies. *If it is adaptive to be chronically stressed, they will evolve in that direction; if not, they will only respond acutely* - Boonstra (2013).

Intriguingly, it was recently demonstrated that chronic stress, either from perceived risk of predation or from experimental corticosterone manipulation, triggers the expression of a larger tail in amphibian tadpoles; larger tails enhance fast-start performance, resulting in increased escape probability when attacked by predators (McCollum & Van Buskirk 1996; Van Buskirk, McCollum & Werner 1997; Relyea 2003). Hence, a classic example of an inducible morphological defence has now been empirically shown to be regulated by physiological stress levels (Maher, Werner & Denver 2013).

Internal stress to external express

Recent decades have seen considerable progress in the development of an integrated framework for the theoretical underpinnings behind the evolution of phenotypic plasticity in anti-predator traits. Further, we have seen plentiful of empirical observations for when plasticity has been favoured in anti-predator traits (Brönmark & Miner 1992; McCollum & Leimberger 1997; Tollrian & Harvell 1999; Brönmark, Lakowitz & Hollander 2011). However, few studies have truly explored the mechanistic underpinnings of phenotypic plasticity, i.e. designed and performed experiments that enable us to bridge the proximate physiological processes that result in the morphological end effects, as Bradshaw discussed more than 55 years ago: "*All changes are physiological in origin, so fundamentally all plasticity is physiological. Where physiological changes have predominantly morphological end effects however, we can talk about morphological plasticity*".

Here, it was recently demonstrated that dopamine may act as a key regulator of phenotypic plasticity in morphological anti-predator traits in *Daphnia* species, a classic invertebrate model system for studying phenotypic plasticity (Weiss *et al.* 2015). Moreover, and as mentioned above, Maher, Werner and Denver (2013) designed a study where they were able to show that chronic stress, defined as enhanced concentration of corticosterone (a key glucocorticoid) is directly responsible for triggering the expression of an inducible defence in amphibian tadpoles. Yet, before the ground breaking study of Maher, Werner and Denver (2013), an earlier experiment had already demonstrated that addition of metyrapone (a glucocorticoid inhibitor) into the water was sufficient to block the expression of morphological defence in predator-exposed tadpoles (Hossie *et al.* 2010). The combined results from these studies are intriguing from several different perspectives. First, they suggest that the glucocorticoid

pathway, i.e. altered stress levels, is the key machinery behind plastic defence regulation. In theory, it has been argued that physiological stress should be considered a logic candidate behind phenotypic alteration, basically since it is well-established that phenotype × environment mismatches often result in a stress response, i.e. the *predation stress hypothesis*, and if such response results in adaptive morphological end effects among plastic prey species, it may explain why stress responses are so multifaceted in nature. Moreover, and more speculative, such mechanism, where chronic stress cause adaptive morphological alteration, may compensate for the maladaptive effects that chronic stress can impose and this may ultimately explain the existence of chronic stress responses in nature (Boonstra 2013; Clinchy, Sheriff & Zanette 2013).

Potential costs of hidden trade-off dynamics

This relatively new insight into the proximate physiological mechanisms of plastic defence regulation, along with diverse evidence for pleiotropic effects on the phenotype of acute and chronic stress, may allow us to find explanations to the puzzling questions of hidden trade-offs associated with adaptive plasticity. For example, and as I test in **paper I**, if predator presence/non-lethal predation risk leads to a chronic stressful situation that subsequently mediates an anti-predator morphology so that the phenotype become more adapted to survive in a high risk environment, then the stress needed to induce the anti-predator phenotype might cause indirect maladaptive effects on immune function (Seiter 2011; Yin *et al.* 2011).

The model system

"Jag trodde i början att det var Gibelio, så urskuren var stjärtfenan och så obetydligt nedböjd var sido-linien, men nog måste man betrakta den som en degenererad Carassius, eller åtminstone på vägen att blifva det – Prof. B. Fries"

"att finna denna öfvergång, blef nu föremålet för en lång och noggrann undersökning" – C. U. Ekström 1838.

In my PhD project, I have used a well-established model system for the study of inducible morphological defences, namely the crucian carp (*Carassius carassius*). This teleost fish provided the first example of an inducible morphological defence among vertebrates (Brönmark & Miner 1992), and has become a classic example of predator-induced morphological plasticity (e.g. Nilsson, Brönmark & Pettersson 1995; Holopainen *et al.* 1997; Pettersson & Brönmark 1997; Hulthén *et al.* 2014). The genus *Carassius* consists of three closely related species: crucian carp (*C. carassius*), goldfish (*C. auratus*) and Prussian carp (*C. gibelio*). Taxonomically, crucian carp and goldfish are closely related, where the latter has been extensively used as a model species in a diverse set of biological research, e.g. stress physiology (Fryer, Lederis & Rivier 1984; Bernier, Bedard & Peter 2004), sensory biology (Hawryshyn & Beauchamp 1985) and phenotypic plasticity (Chivers *et al.* 2008). The crucian carp is widely distributed across Europe and central Asia; in ponds, lakes and rivers, as well as in the brackish water of the Baltic basin (Kottelat & Freyhof 2007). In nature, crucian carp occur in two different morphs, a dissimilarity so distinct that it for long was classified as two separate species (Ekström 1838), see Figure 5. In general, crucian carp are tolerant to adverse abiotic factors, such as varying temperature and oxygen depletions, but, in contrast, it is very sensitive to biotic factors, such as interspecific competition or predation from piscivorous fish, e.g. pike (*Esox lucius*, see front cover) (Holopainen, Tonn & Paszkowski 1997). This disparity in tolerance to different stressors is clearly recognized in earlier publications, where titles such as "*Tales of two fish: the dichotomous biology of crucian carp in northern Europe"* have been used (Holopainen, Tonn & Paszkowski 1997).

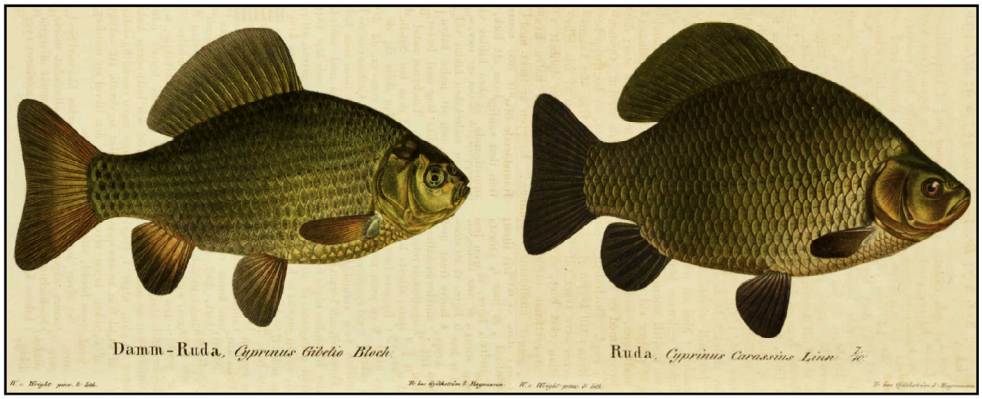


Figure 5. Painted illustrations of the two phenotypes of crucian carp by Wilhelm von Wright and published in the nowadays classic book Skandinaviens fiskar. Back then (anno 1837), long before the concept of phenotypic plasticity was coined, the two morphs were distinguished as separated species, to the left: Damm-ruda i.e. pond crucian carp (Cyprinus gibelio Bloch) and to the right: Ruda i.e. crucian carp (Cyprinus carassius). A quite spectacular thing is that the darker body colouration in the predator-induced phenotype was evident already then, in these beautiful paintings. Yet, this difference in body colouration was not described until year 2020, when I tested the effect of cortisol and predator-exposure on phenotypic trait alterations (p**aper II**).

Why crucian carp?

To illustrate why this is an excellent model system for studying evolutionary hypotheses on phenotypic plasticity in anti-predator traits, I will briefly relate the four factors that, according to the general consensus, favour the evolution of plasticity (see earlier section) to our recent understanding of the evolution of inducible defences in crucian carp:

I. The inducible defence is proven effective

The most famous defence strategy that crucian carp express when exposed to chemical cues from predatory fish is the induction of a deeper body shape (Brönmark & Miner 1992). This extreme morphological plasticity in response to predation risk was, as mentioned above, the reason for why the two morphs for long were viewed as two separate species (Ekström 1838). A deeper body shape directly reduces predation risk, as deep-bodied individuals constitute less desirable prey and are more difficult to handle for gape-size limited predators (Nilsson, Brönmark & Pettersson 1995; Nilsson & Brönmark 2000) and also show increased escape performance via enhanced locomotor capacity (Domenici *et al.* 2008). Furthermore, in **paper II** I show that crucian carp alter their body colouration towards a much darker appearance when co-existing with a live predator, which, along with plasticity in diel activity followed by cryptic defence plasticity in eye morphology (**paper V**), may produce an integrated anti-predator phenotype in crucian carp (see further discussion below).

II. There are reliable cues

In comparison to the terrestrial world, many aquatic environments hold poor light transmission along with high habitat complexity. Such characteristics directly reduce visual capacity, which from an organismal perspective may cause maladaptive behaviours, e.g. swimming into a risky area where a predator may attack. Therefore, evolution has taken advantage of the soup of chemicals making up the natural world below the surface and have favoured the development of very precise communication and recognition of the environmental properties via chemical cues (Brönmark & Hansson 2012). Numerous studies have shown that aquatic organisms of different taxa use chemical cues to cope with intra- and interspecific interactions in an adaptive manner (Brönmark & Hansson 2012; Mitchell, Bairos-Novak & Ferrari 2017). As will be discussed below, crucian carp is not an exception. However, before going into the knowledge of the cues that crucian carp use to evaluate changes in risk in order to fine tune the expression of their distinctive phenotypes, I should mention that the chemicals that induce biological responses are, depending on their origin and mechanism, classified into different functional groups. I will briefly mention this classification below, see Brönmark and Hansson (2012) and Mitchell, Bairos-Novak and Ferrari (2017) for more details.

First, we have the *kairomones,* chemicals released by one organism, the sender, which, when detected by another organism, the receiver, results in some adaptive benefits for the receiver but not for the sender. Here, the sender is often a predator and the receiver a prey, and, hence, kairomones can further be characterised into *predator odour* i.e. chemicals directly derived from the predator *per se* and *diet cues*, i.e. chemicals released when specific prey are consumed by the predator. In addition to kairomones, we have *chemical alarm cues* (also known as *Schreckstoff*) which are released as a by-product from bodily damage after e.g. a predator attack. These alarm cues are produced by specific cells, called club cells, that are located in the upper layer of the epidermis. The chemical compounds in club cells cannot be released voluntarily by the prey, instead they leak when epidermal tissue is damaged, and detection is therefore a reliable source of information about a present and foraging predator (Ferrari, Wisenden & Chivers 2010). As a parenthesis, research suggests that the primary function of these specialized cells are immune function by supporting epidermal integrity towards diverse environmental pathogens. The ecological role as anti-predator function has evolved secondarily, when selection has favoured detection, followed by adaptive responses. At last, and in contrast to chemical alarm cues, some species have evolved more active ways to signalling an impending danger. These so-called *disturbance cues* differ from chemical alarm cues by not being a by-product, instead they are released to deliberately alert nearby conspecifics of a potential danger e.g. a nearby predator.

Earlier studies have demonstrated that crucian carp show strong behavioural and morphological responses to chemical cues, already at extremely low concentrations (Pettersson, Nilsson & Brönmark 2000). First, Brönmark and Pettersson (1994) experimentally examined the source of the cues that elicit the defended morphology and they concluded that chemical cues are enough and visual cues are not necessary to elicit a change in morphology. Furthermore, they showed that predators only induce a deeper body shape in crucian carp when going on a strict crucian carp diet and not when feeding on macroinvertebrates. Hence, this experiment clearly illustrates that it is either diet cues from the digestive process or chemical alarm substances derived from injured conspecifics that triggers the facultative expression of its morphological defence. Some years later, it was shown that the piscivorous diet must contain prey having alarm cells, i.e. water containing the chemical compound from pike feeding on swordtails (*Xiphophorus helleri*) did not trigger any behavioural response (Pettersson, Nilsson & Brönmark 2000). Interestingly, the same study demonstrated that starved predators (pike) also trigger a fright response, equally strong as recently fed pike predators. This indicates that crucian carp may use both kairomones, such as predator odour, and chemical alarm cues from closely related species with club cells, to respond behaviourally to potential danger. However, we know from more recent experiments that, at least for the behavioural fright response, it is enough with supernatant from skin extracts, i.e. chemical alarm cues from club cells (Lastein *et al.* 2008). Moreover, we know that crucian carp respond to extremely low concentrations of chemical cues.

To sum, crucian carp have evolved a capacity to detect reliable cues that allow them to reliably evaluate the prevailing predation risk.

III. Extreme environmental heterogeneity

Predation is not the only selective agent as this species has evolved some very unique tolerance against. In fact, the ultimate reason behind the spectacular defence plasticity against predation lies in another, highly unique, adaptation i.e. the capacity to survive complete anoxia for a significant amount of time (Nilsson 1990). This adaptation allows survival in small and shallow water bodies across the northern hemisphere, where ice and snow may block photosynthesis for months, rendering anoxic conditions that are impossible to survive without such an adaptation (Hyvärinen 1985; Nilsson 2001). Total winterkill of their natural enemies, such as pike and perch (*Perca fluviatilis*) is a common phenomenon in nature (Brönmark & Weisner 1992). However, waterscapes (the wet synonym to "landscape" in terrestrial environments), often enable dispersal of individuals between ponds and lakes by interconnected streams or during flooding. Such dispersal favours the recolonizations of piscivorous species into periodically anoxic habitats. Therefore, by being extremely tolerant toward anoxia in combination with a relatively long life-span (Tarkan *et al.* 2016), individual crucian carp may experience

multiple stochastic environmental shifts, where predation as a regime of selection can vary in a bimodal way.

IV. There must be associated costs

At high densities and in the absence of predators, crucian carp with the morphologically defended phenotype show reduced growth rates as compared to the undefended morph (Pettersson & Brönmark 1997), indicative of a resource-dependent cost of the inducible defence, possibly coupled to morphology-associated cost differences in sustained swimming performance (Pettersson & Brönmark 1999; Pettersson & Hedenström 2000). Moreover, crucian carp show a considerable inter-individual variability in the magnitude of morphological defence expression. This has partly been linked to individual differences in behavioral traits, where bolder individuals, assumed to be exposed to greater risks, also show a more pronounced morphological defence (Hulthén *et al.* 2014). This inter-individual difference in morphological defence expression suggest a trade-off between behavioral and morphological anti-predator traits. Moreover, in **paper I** and **paper IV** I investigate and discuss other aspects of trade-offs in relation to the morphological defence plasticity. Briefly, in **paper I,** I search for potential trade-offs between defenses against different natural enemies; i.e. do investment into a morphological defence against predators' trade-off with investments into immune defence against pathogens? Furthermore, in **paper IV** I suggest a strong trade-off between reproductive investment and investment into the anti-predator phenotype, i.e. sex-specific trade-offs.

Aims of the thesis

The initial focus of this thesis has been founded on the current gap in our knowledge regarding the proximate, physiological mechanisms controlling phenotypic plasticity in morphological anti-predator traits. I have here employed a hypothesis of stress being the machinery behind morphological defence regulation (**paper II-IV**). Following this theoretical framework, I further examine classic resource allocation trade-offs to search for hidden physiological costs coupled to perceived predation risk and investment into a morphological defence (**paper I**). Lastly, based on personal observations during the initial laboratory experiments, I tested hypotheses about phenotypic plasticity in additional traits such as body colouration (**paper II**), diel activity and eye morphology (**paper V**).

From physiology to morphology

In **Paper I**, I experimentally test for potential costs of predator exposure on immune function in crucian carp, i.e. costs caused by inducing the morphological anti-predator traits resulting in a trade-off between resources available for investments in defence traits *versus* immune function. Second, I expand on earlier work examining the underlying physiological processes of vertebrate plasticity, and tested if glucocorticoid concentration (cortisol) is the key regulator of morphological defence expression in my model system (**paper II**) and if so, would the application of a psychoactive drug (SSRI, fluoxetine) that targets the serotonergic system block the morphological response upon long-term predator-exposure via an inhibition of the stress-axis (**paper III**). Earlier studies have shown that serotonin stimulates the release of glucocorticoids during short-term SSRI exposure, whereas under chronic exposure an increase in serotonin levels is linked to reduced stress sensitivity, i.e. diametrically opposite effects (Winberg & Thörnqvist 2016). Hence, I further ask how fluoxetine would influence anti-predator behaviour measured as the propensity of individual risk-taking behaviour, by quantifying boldness after short- and long-term exposure (**paper III**). Based on earlier studies showing sex-specific differences in stress coping styles along with the general consensus of sex-specific differences in reproductive investments, I examined if male

and female crucian carp differed in (i) maximum morphological defence expression (field data) and (ii) if there are sex-specific differences among predator naïve crucian carp in the plastic response towards a sudden increase in non-lethal predation risk (laboratory experiment) (**paper IV**).

Specifically, I ask the following questions:

Physiological trade offs

- According to the *predation stress hypothesis*, predators elicit a significant increase in prey stress levels. Stress, if being constantly present, may result in a resource trade off with other important physiological functions such as immune function. So, does predation risk alter key aspects of innate immune function? **Paper I**.
- Do investments into a morphological anti-predator defence result in a trade-off with immune function quality? **Paper I**.
- Do females, who invest more into reproduction, show less morphological response to predator exposure? **Paper V.**

Proximate mechanisms

- If chronically high stress levels are underlying the expression of external anti-predator traits (body depth and body colouration), can we then induce an anti-predator phenotype by experimentally manipulating cortisol levels? **Paper II.**
- Following the stress hypothesis, can we block the anti-predator phenotype in predator-exposed individuals with a cortisol inhibitor? **Paper II.**
- The neuroendocrine system is a target for endocrine disruptors, such as psychoactive pharmaceuticals, which are common aquatic contaminants. We hypothesized that exposure to an antidepressant pollutant, fluoxetine, influences the physiological stress response in our model species, crucian carp, affecting its behavioural and morphological responses to predation threat. **Paper III**.
- Can we find support for the proximate mechanism behind the observed sex-specific variation in the anti-predator phenotype from differences in expression profile of the POMC gene? **Paper IV**.

A suite of phenotypically plastic defence traits

When I was running my first experiments I noticed that crucian carp altered their body colouration when exposed to a predator (pike). I therefore used this observation to examine melanin-based plasticity in response to non-lethal predation risk and physiological stress (cortisol manipulation), see **paper II**. Moreover, it is known since earlier that crucian carp become relatively inactive and spend less time foraging when there is a risk of predation (Pettersson & Brönmark 1993; Holopainen *et al.* 1997; Andersson, Johansson & Soderlund 2006). Yet, after some late-night visits to my lab I noticed that experimental subjects reared with a predator showed contrasting behaviours when the light was off compared to when the light was on. This led me to an idea that crucian carp might have evolved plasticity in circadian rhythm/diel activity to create an adaptive mismatch towards their main predator, pike, which is a visually foraging predator and, thus, mainly diurnal (Skov & Nilsson 2018). However, earlier studies on diel activity changes in crucian carp showed contrasting results. For example, a field study from Finland showed some support for my hypothesis of predator-induced plasticity in diel activity as crucian carp existing in high predation sites were more nocturnal than crucian carp in low predation sites (Tonn, Paszkowski & Holopainen 1989). Conversely, a laboratory experiment found no evidence for a shift towards nocturnality in crucian carp exposed to chemical cues from pike (Pettersson et al., 2001). Instead, it was found that crucian carp exposed to pike cues demonstrated an aperiodic activity pattern across the whole experimental period (11 days). But, since I repeatedly noticed the contrast in day *versus* late-night behaviour in my experimental subjects, I decided to test the hypothesis of adaptive diel plasticity but using a long-term exposure experiment, that better would mimic natural conditions, see **Paper V**. Moreover, since a nocturnal lifestyle would directly reduce visual capacity, if not compensated by sensory plasticity, I investigated if crucian carp alter their eyes when co-existing with predatory pike, **Paper V.** A major current goal in evolutionary biology is to understand the ecology and evolution of vertebrate vision. For most animals, vision is a key sensory system for foraging and predator avoidance (Land & Nilsson 2012). Given its importance, an astonishing diversity of eye morphologies and visual strategies have evolved across taxa and environments (Schwassmann & Kruger 1966; Land & Nilsson 2012; Nilsson *et al.* 2012). While numerous studies have unravelled how predation risk can affect prey eye and pupil size at micro- and macro-evolutionary scales (e.g. Nilsson *et al.* 2012; Banks *et al.* 2015; Beston & Walsh 2019), few have examined intra-individual responses in eye morphology to changes in key environmental cues, including predation risk.

Specifically, I ask the following questions:

- Since the melanocortin system and the stress axis interact and are influenced by the POMC gene, predator-induced stress should result in a darker body colouration from more melanocortins that should stimulate melanin-based colouration. In **paper II**, I ask if that is the case i.e. do predator exposure induce a darker body colouration?
- For prey, it is important to avoid interactions with predators. I therefore, in **paper V**, ask whether diurnal and predator-naïve crucian carp can adaptively tailor their diel activity patterns by switching to a nocturnal lifestyle when being exposed to a diurnal predator? Such diel plasticity would, for the prey, result in direct fitness benefits from the reduced risk of predator-prey interactions.
- Predation has been linked to selection for larger eye size in prey organisms. A larger eye can hold a larger pupil, and, hence, results in positive effects on prey and predator detection, particularly under dim light. In **paper V**, I found that crucian carp shift diel activity towards a more nocturnal lifestyle, and based on this finding I asked whether crucian carp can plastically compensate for the poor light regime by increasing eye and pupil size? **Paper V**.

Methods

In general

I have used wild-caught and previously predator-naïve crucian carp caught from three small lakes in the vicinity of Lund, southern Sweden. Fish were caught with fyke nets and baitfish traps (Figure 6), and immediately transported to experimental facilities at Lund University where they were acclimatized to laboratory conditions in large tanks (aerated and filtered) and fed a varied diet consisting of chironomids, water fleas, shrimp mixes and commercial carp pellets *ad libitum*. In order to enable identification of individuals throughout the experiments, a passive integrated transponder tag (HDX, Oregon RFID, size: 12.0 mm long & 2.12 mm diameter, weight 0.1 g, see Figure 7) was surgically implanted into the abdominal cavity of each crucian carp prior to the experiments, following (Skov *et al.* 2005). In all experiments, I have used live predatory pike individuals to experimentally manipulate non-lethal predation risk within the experimental arenas. All pike were caught by electrofishing in lake Krankesjön, southern Sweden, transported to the experimental facilities at Lund University, acclimatized to laboratory conditions and fed a strict diet of crucian carp one to two times per week. For the experiments in **paper I - V**, I set up 24 aquaria (152 L; 95x40x40 cm, (Figure 8), each divided into two compartments of equal size by a perforated transparent acrylic glass partition. This set-up allowed crucian carp in the predator presence treatment to perceive both visual and chemical cues from pike, and to respond to the chemical alarm substances released from pike feeding on crucian carp in the experimental arenas. To reduce stress among fish included in the experiments, artificial vegetation were used in both sections of each aquarium, and in order to prevent visual interactions between replicates, three sides of each aquarium were externally covered with a black plastic film. In general, all laboratory experiments were conducted at a constant temperature (18°C) and a 12:12 hr light:dark regime.

Figure 6. All crucian carp individuals were caught in ponds or small castle lakes in the vicinity of Lund, Sweden. Either by the use of (A) fyke net or (B) smaller baitfish traps. Crucian carp populations often thrive when no predators are present (C) so some field assistance sorting fish was valuable.

Figure 7. Illustration of a PIT (Passive Integrated Transponder)-tag that I surgically implanted into the stomach cavity of experimental subjects in each laboratory experiment to enable identification of individuals throughout the course of the experiments.

Figure 8. The general experimental set-up that I have employed in **paper I-V**. Each aquaria (152 L; 95 cm x 40 cm x 40 cm) was divided into two compartments of equal size by a perforated transparent acrylic glass partition. This set-up allowed crucian carp in the predator presence treatment to perceive both visual and chemical cues from pike.

Specifics

Paper I

The aim was to study whether predator exposure affects immune function in crucian carp, and if the degree of expressed morphological defence against predation is traded off against immune function. First, all fish (n=20, 105-118 mm, standard length) were photographed and weighed after either being exposed or non-exposed to a live predatory pike for almost 250 days. Twenty-four hours later, I anaesthetized all individuals with benzocaine and collected a blood sample by cardiac puncture using a heparinized 30-gauge syringe. These blood samples were subsequently used to examine baseline immune function.

Nineteen days after the baseline measurement, an immune response were experimentally triggered via a lipopolysaccharide (LPS) challenge, a method known to induce an immune activation without the direct negative effects associated with an actual infection (Novoa *et al.* 2009; Hegemann *et al.* 2013). This was done via intraperitoneal injection of LPS just above the pelvic fin. Subsequently, I collected a blood sample 13-hours post LPS injection, i.e. during the assumed acute-phase response (Swain *et al.* 2008; Hegemann *et al.* 2013). In addition, because ectotherm fish might have a slower, i.e. longer, immune response than endotherm animals, I also took another blood sample seven days post injection to test for potential long-term consequences (Sköld-Chiriac *et al.* 2014). No mortality occurred during the sampling period.

From the blood samples, three parameters of the innate immune function were assayed and compared between treatments and individuals: 1) *lysis* and 2) *agglutination* reflect responses to foreign cells driven by the complement system (lysis) and natural antibodies (agglutination) (Matson, Ricklefs & Klasing 2005; Uribe *et al.* 2011), and 3) *haptoglobin*, a protein of the acute-phase response. Haptoglobin release is regulated by the liver and often activated during the onset of infection (Murata, Shimada & Yoshioka 2004; Quaye 2008).

Morphological defence expression was quantified from the digital photos.

Paper II

Focusing on the hypothesis of physiological stress as the underlying mechanism behind plastic trait expression, I exposed predator naïve crucian carp (n = 144, body weight: 9.3 ± 0.9 g; mean ± SD) to contrasting predator environments (presence/absence of pike) as well as to experimental manipulation of physiological stress via endogenous implants containing either cortisol or a cortisol inhibitor (metyrapone).

Implants were injected intraperitoneally with a 23-gauge needle inserted posteriorly to the pectoral fin. After each injection, I immediately placed a small ice bag on the location of the injection to enhance solidification of the implants, following previous work (e.g. Bernier & Peter 2001). I employed previously used dosages of both cortisol and metyrapone; 150 μg cortisol/g body weight (BW) and 200 μg metyrapone/g BW. All substance deliveries, including the sham injections (containing plain cocoa butter), were individually adjusted to correspond to an intraperitoneal injection of 10 μl implant solution/g BW.

Treatment exposure lasted for five months in a controlled laboratory environment and then key aspects of the phenotype (body colouration and body morphology) were quantified using digital photography and melanophore analyses from scale samples.

Paper III

Psychoactive drugs, such as fluoxetine, may influence the stress sensitivity of our model species, crucian carp, affecting its behavioural and morphological responses to predation threat. Therefore, I exposed crucian carp (n = 144, total length: 11.56 ± 0.03 cm; mean ± s.e.) to three different concentrations (0, 1 and 100 μg L^{-1}) of fluoxetine in both the absence and presence of a live predatory pike. Treatment exposure lasted for six months in a controlled laboratory environment, and then key aspects of the phenotype (boldness and body morphology) along with chemical analyses of fluoxetine concentrations and its active metabolite norfluoxetine in blood plasma of experimental subjects were measured.

Paper IV

To test the hypothesis of an energetic trade-off between resources partitioned to reproduction *versus* inducible defences, I first performed a field study where I asked whether the variation in morphological defence expression (body depth) among crucian carp observed in two wild lake populations could be explained by sex differences. Next, I set up a laboratory experiment where I exposed crucian carp to control or predator (a live predatory pike) treatments and quantified sex-specific predator-induced changes in body depth.

A comprehensive method of landmark-based morphometrics were used to examine potential differences between the sexes. In total, I digitized 11 homologous landmarks on each fish using tpsDig2 (Rohlf 2017) from which Generalized Procrustes Analysis were used to scale, rotate, and superimpose the landmarks of all fish (field and laboratory experiment).

Further, changes in stress-related glucocorticoid release, suggested to affect inducible defence expression (Hossie *et al.* 2010; Maher, Werner & Denver 2013; Vinterstare *et al.* 2020), is driven by the expression of the polypeptide precursor pro-opiomelanocortin (POMC) gene (Harno *et al.* 2018). Therefore, I dissected the brains of a subsample of fish (n tot = 14) at the end of the laboratory experiment. From these whole-brain samples, RNA were extracted and subsequently assayed for differential gene expression analysis of the POMC gene.

Paper V

To study potential plasticity in eye morphology and diel activity patterns I set up two experiments, both involving wild-caught and previously predator-naïve crucian carp (eye plasticity: n = 96, size range 83-93 mm; diel activity: n = 70, size range 83-110 mm)

Eye plasticity was quantified as trait changes in overall eye and pupil size at the level of individuals from digital photographs. I followed earlier studies and measured the widest part of the eye and pupil in the horizontal plane (Beston, Wostl & Walsh 2017; Svanbäck & Johansson 2019). In addition, standard length was measured (the distance between the tip of the snout to the end of the last scale anterior to the caudal fin) of all fish to account for potential body size differences and enable calculation of relative eye and pupil size.

In a behavioural experiment, I further examined the effect of predation risk on diel activity patterns in crucian carp. During the diel activity trials, I employed different light regimes simulating natural conditions: (i) day (7:00-19:00; ~540 lx), (ii) evening (19:00-20:58; ~18 lx), (iii) night (21:00-5:00; 0 lx) and (iv) dawn (5:00-7:00; ~18 lx).

Digital infrared scouting cameras (UOVISION UV572 12 MP HD, infrared wavelength: 960 mm) were used to allow monitoring of fish activity also under low light conditions. All fish were fed 24 hours before each trial started to standardize hunger levels prior to activity measurements.

Lastly, the maximum distance for visual detection of dark objects were modelled according to theory developed for aquatic vision (Nilsson, Warrant & Johnsen 2014). The pupils were assumed to be circular, and the estimated means of the pupil diameters from each treatment group (predator-exposed or control) were used to compare the visual range, although we modelled visual performance for the complete pupil range of 0-4 mm. The maximum distance (visual range) for detection of circular black targets were modelled for diameters of 1 mm (planktonic prey), 10 cm (predators) and an intermediate value of 1 cm. This was done for different light regimes, i.e. daylight, twilight and starlight.

Main results and conclusions

Physiological costs

In **paper I**, I found that perceived predation risk altered key aspects of the innate immune function. From the three quantified immune variables, I could conclude that non-lethal predation risk affected the immune function of crucian carp. More specifically, I found that predator exposure resulted in lower baseline values of haptoglobin levels and complement activity and, in contrast, resulted in a higher mean of natural antibody titres compared to individuals reared under predator-free conditions. Furthermore, I found that predation risk resulted in a generally weaker immune response when experimentally challenged with a mimicked bacterial infection (LPS injection). The joint results clearly demonstrate evidence of predator-induced changes in immune function. However, if the proximate explanation for these changes in immune function are caused by an increase in glucocorticoid concentrations from predator-induced stress has to be examined in future studies.

Intriguingly, I found multiple correlations between immune variables and the degree of expressed morphological defence. Here, based on the hypothesis of trade-off dynamics between the two examined defences, I predicted the slope of correlation to be negative. However, even though both baseline immune function and the ability to mount an immune response in individual fish correlated with the individual expression of the morphological defence, these relationships were not consistently supportive of a general trade-off among defences. Instead, I suggest that these results better illustrate individual variation in the capacity to handle predation risk, where some fish seems to cope significantly better than other conspecifics. Furthermore, it can be argued that the results from **paper I** goes hand in hand with the personality-morphology relationship previously demonstrated in crucian carp. Bold individuals express a deeper, predator-induced body morphology compared to shy individuals (Hulthén et al. 2014). Along with my findings of a relatively high innate immune function among deep-bodied individuals, I suggest that phenotypic compensation may explain the results. Boldness are per definition linked to explorative behaviour and higher activity levels, which ultimately should increase the risk of encountering predators and pathogens. Moreover, as discussed in **paper I**, bold and fast-living organisms should rely more on innate

immunity, since this part of the immune system is cheaper, develops faster and is more general against pathogens than adaptive immunity. However, since I in **paper IV** found strong sex-specific differences in the morphological anti-predator response, I strongly suggest that future studies have to include sex as a factor when examining trade-off dynamics within this system.

In **paper IV**, I demonstrate clear evidence for sex-specific variation in morphological defence expression. The observed pattern of males investing more into the morphological anti-predator defence was evident both among wild crucian carp originating from high predation sites and after a manipulation experiment where predator-naïve individuals where exposed to a pike under controlled laboratory conditions. Among vertebrates, female gametes are generally much more expensive as compared to male gametes, resulting in divergent resource allocation among sexes into e.g. reproduction and survival (Zera & Harshman 2001). Such trade-off may explain why male fish display a more pronounced anti-predator phenotype and if not compensated by adaptations in e.g. anti-predator behavior, female may suffer from relatively high predation risk.

To conclude, I found that:

- The mere presence of predators has the capacity to cause potentially important changes in immune function quality of prey. These changes may be caused by a chronic increase in glucocorticoid concentrations, following the prediction for the predation stress hypothesis. Furthermore, I found that the baseline value of all three immune variables strongly correlated with the individual magnitude of expressed morphological defence against predation. Yet, I did not find support for a trade-off between defences (protection against divergent enemies i.e. pathogens and predators). Instead, I argue that my findings can best be explained from individual fitness and pace-of-life perspectives where some individuals can cope with predation risk significantly better than other conspecifics.
- Males show a significantly more pronounced morphological defence against predation. I suggest that this sexual dimorphism in the anti-predator phenotype may be caused by sex-specific trade-off dynamics where females invest more into reproduction, resulting in less resources for anti-predator protection.

Proximate mechanisms

Considerably few studies have explored multiple trait changes and the underlying physiological mechanisms that modulate the integrated defence phenotype, i.e. from perception of predator cues to physiological changes leading to the expression/inactivation of traits known to influence predator avoidance. This is a critical gap in our knowledge, and if we are to understand the puzzle of phenotypic plasticity it is critical to elucidate these questions. In **paper II**, I test the hypothesis of physiological stress as the regulatory machinery behind morphological defence expression. Basically, I employ a very similar experimental design as was recently used when examining the proximate mechanism behind defence regulation in another system, i.e. phenotypically plastic amphibian tadpoles. However, whereas Maher, Werner and Denver (2013) found that it was the end product of the stress-axis, i.e. enhanced levels of a glucocorticoid (corticosterone), that triggered the expression of amphibian defence morphology, I found the opposite, namely that individuals which were exposed to a natural predator became darker and induced a deeper body shape, whereas cortisol-treated fish showed lower expression of a deep body morphology with a more pale body colouration as compared to sham-treated fish. First, this suggests that amphibian tadpoles and crucian carp have evolved different pathways for their defence regulation. Second, it suggests that both model systems show evidence for stress being the responsible mechanism of plastic defence regulation. However, instead of an increase in glucocorticoid concentrations *per se*, my results from **paper II** suggest that the overall effect of enhanced stress levels may cause the expression of morphological anti-predator traits. The first argument is based on the findings of the dramatic darkening in body colouration among predator-exposed fish. Here, detailed analysis revealed that the mechanism underlaying the predator-induced change in body colour was a higher melanophore density, i.e. increased melanogenesis. Increased melanisation is costly, and among teleost fish known to be a result of increased melanin biosynthesis from melanocortin peptides such as ACTH and α-MSH which are under direct regulation of POMC gene expression (Aguilera 1994; Sapolsky, Romero & Munck 2000; Slominski *et al.* 2000; Leclercq, Taylor & Migaud 2010). The second argument is founded on the theory of the first, where the negative feedback cycle of the vertebrate stress axis is directly regulated by a suppression of POMC gene expression(Drouin *et al.* 1989). Hence, such down regulation should, theoretically, result in a dampened melanin biosynthesis which can explain the findings of contrasting trait trajectories among cortisol treated fish, at least the paler body colouration. To sum, **paper II** illuminates a link between stress physiology, colouration and morphological defence

expression and highlights the far-reaching impacts of predation risk on prey plastic phenotypes.

As discussed (a lot), the neuroendocrine regulation of stress levels should be a logic candidate for plasticity in anti-predator traits. However, this system is also a target of neuroendocrine disruptors, including environmental contaminants such as pharmaceuticals. One class of pharmaceuticals of special concern in this context is the selective serotonin re-uptake inhibitors (SSRIs), such as the commonly prescribed fluoxetine (marketed as Prozac^{TM}). SSRIs are explicitly designed to modulate human physiology and behaviour, but as the target receptors and physiological pathways have been evolutionary conserved among vertebrates, it is highly likely that serotonergic drugs also affect non-target organisms, where aquatic organisms are particularly exposed. In **paper III**, I found that a high dose of fluoxetine exposure dramatically changed the behaviour of crucian carp. After short-term exposure to a high concentration of fluoxetine, fish became relatively shyer whereas unexposed and low-dose exposed fish shifted towards increased boldness. Intriguingly, the initial and strong effect of high-dose FLX treatment on crucian carp behaviour was not present at the end of the experimental period, instead all treatments showed the same increase in average boldness.

Second, I found that fluoxetine exposure altered the expression of the relative body depth i.e. the inducible morphological defence. The effect of fluoxetine on body shape was context dependent, i.e. the high FLX treatment strongly influenced morphology of fish regardless of the prevailing risk of predation, whereas the low FLX treatment influenced body shape of fish in the presence, but not in the absence, of a predator. This resulted in significantly different predator-induced morphologies within each FLX treatment, potentially pointing to fitness consequences of environmentally relevant concentrations of an SSRI pollutant. Based on the findings in **paper III**, I struggle to draw any clear conclusions about the proximate mechanism behind the morphological plasticity of crucian carp. A discussion about the proximate mechanisms becomes even more complex in the light of the findings in **paper IV**. Here, I show that male express significantly more morphological defence than females and that males show a higher degree of predator-induced plasticity than females. Subsequently, from our gene expression analyses, predator-exposed males were found to have an upregulation of the POMC gene. This suggests a higher level of predator-induced stress in male fish, and thus, that such enhanced stress may drive the sexual dimorphism in the anti-predator phenotype. However, teleost fish have evolved multiple copies of the POMC gene and we did only detect this up-regulation in males for one of three POMC copies. Therefore, future studies should try to disentangle the proximate differences between

POMC copies and expand on this work with increased sample sizes along with a temporal perspective, i.e. to include more time points.

To conclude, I found that:

- The phenotype of individuals carrying cortisol implants did not mirror the phenotype of predator-exposed fish but instead exhibited opposite trajectories of trait change: a shallow-bodied morphology with a lighter body coloration as compared with sham-treated fish. I suggest that the proximate explanation of these findings are best explained from the negative feedback system of the stress axis, where excessive levels of cortisol would result in a suppression of POMC which would explain the reduced melanocortin concentrations.
- Metyrapone (a cortisol inhibitor) did not influence the phenotype of fish, i.e. neither body depth nor body coloration differed between this group and predator-exposed fish.
- Fluoxetine effects on morphological plasticity were context dependent as a low dose only influenced crucian carp body shape in pike presence. A high dose of fluoxetine strongly influenced body shape regardless of predator treatment. Our results highlight that environmental pollution by pharmaceuticals could disrupt physiological regulation of ecologically important inducible defences.
- Males express more morphological defence in the wild and show a higher degree of predator-induced plasticity in laboratory settings. Further, a candidate stress gene (POMC) was found to have a higher expression in predator-exposed males as compared to non-exposed male controls. At least hypothetically, such sex-specific differences in predator-induced POMC expression may explain the clear sexual dimorphism as observed in the anti-predator phenotype.
- I suggest that sex-specific anti-predator responses may be an important, yet underappreciated, component underlying inter-individual differences in the expression of inducible defences, even in species without pronounced sexual dimorphism, as the crucian carp.

A suite of phenotypically plastic defence traits

In addition to the extreme plastic control of relative body depth, I here present novel data that strongly suggest that crucian carp are capable of simultaneous trait alteration in a suite of behavioural, sensory and morphological traits as a response to perceived predation risk. First, in **paper II** I show that crucian carp induce a dramatically darker body colouration (see Figure 9) when co-existing with a live predatory pike under controlled laboratory conditions. Body colouration is a key trait, suggested to influence processes as diverse as thermoregulation, photoprotection, social signalling and predator avoidance. (e.g. crypsis and background matching). I suggest that the darker body colouration in crucian carp may act as an inducible defence against predation; a conspicuous signal of the morphological defence along with crypsis when synchronized with their nocturnal lifestyle, as shown in **paper V**.

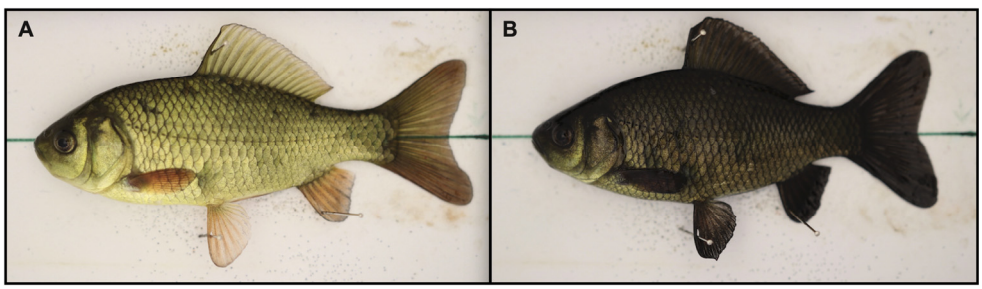


Figure 9. The colour variation between (A) a non-exposed control fish and (B) a predator-exposed fish. Although these photographs were taken after approximately six months of treatment exposure, the change in body colouration is much more rapid. According to personal observations, the darker body are significantly evident already within one week of predator-exposure. However, the fact that the darker phenotype are persistent over time may suggest a situation where predator-exposed fish are experiencing a chronically high stress level. Such situation would per defintion be demonstrated in an upregulated POMC-gene and result in higher concentrations of POMC-derived melanocortins, which subsequently would stimulate pigment production causing a darker body colouration.

Vision allows a reliable window to the world, and earlier studies have examined the selective role of predation for eye evolution (e.g. Nilsson *et al.* 2012; Banks *et al.* 2015; Beston & Walsh 2019; Svanbäck & Johansson 2019). However, in **paper V** I show that the crucian carp, a freshwater fish, expresses a plastic increase in the size of the pupil when exposed to a visually foraging and diurnal predator (pike). This result is novel, as the pupils of teleost species (the majority of fishes), have, hitherto, been described as fixed and unable to respond to rapid changes in environmental conditions. This is because teleost species in general lack a sphincter pupillae muscle of the iris (Helfman & Helfman 2009; Douglas 2018), so how crucian carp actually increases their pupil size would be an intriguing question for future studies. Using a modelling exercise, we further demonstrate that the increase in pupil size among predator-exposed fish (as

compared to predator-free controls) considerably increases their visual range, resulting in a 6% larger visible water volume for prey detection in dim light. Furthermore, our model also reveals that the visual range of the predators used in our experiments decrease to about half from sunlight to twilight, and a further reduction to 12% at starlight. This implies that the water volume covered by vision is 0.125% in twilight and 0.002% in starlight, as compared to sunlight. Hence, being a teleost lacking a sphincter pupillae muscle of the iris, the crucian carp's ability to plastically induce pupil enlargement should render fitness advantages. Specifically, since the visual conditions of freshwater habitats are characterized by high absorption and scattering of downwelling light (Holopainen, Tonn & Paszkowski 1997), where a larger pupil size significantly improves contrast detection and visual range (Nilsson *et al.* 2012; Veilleux & Kirk 2014; Caves, Sutton & Johnsen 2017).

Moreover, in **paper V,** I also demonstrate that predator-exposed crucian carp shift to a more nocturnal lifestyle. This finding is in line with an earlier field study (Tonn, Paszkowski & Holopainen 1989), but in contrast to a laboratory experiment testing how predator presence influence diel activity in crucian carp (Pettersson et al., 2001). This earlier study was examining the diel activity pattern over the course of days and as earlier mentioned found that the presence of pike cues resulted in an aperiodic activity pattern. Even though the study did not show any sign of adaptive calibration of the diel activity, my results in **paper V** do. I suggest that the contrasting results is best explained from a time-dependent context, i.e. crucian carp seem to need some time to adaptively change their diel activity pattern. To speculate, it might be that the observed pupil size increase needs to happen first to allow efficient foraging also under dark conditions when predation risk from diurnal and visually oriented predators is reduced.

I suggest that the predator-induced trait plasticity observed in **paper II** and **paper V** should be adaptive and may act in synergy to produce an integrated anti-predator phenotype.

To conclude, I found that:

- Predator-exposed fish expressed a deeper-bodied phenotype and darker body coloration as compared with non-exposed individuals.
- The darker body coloration may act as an inducible defence against predation, via a conspicuous signal of the morphological defence or by crypsis towards dark environments and a nocturnal lifestyle.
- Crucian carp show phenotypic plasticity with regards to pupil size, but not eye size, as pupil size increased when exposed to predators (pike).
- Predator-exposed crucian carp shift from diurnal to nocturnal activity.

Additional observations, challenges and suggestions for the future

A tropical example of sexual dimorphism in stress physiology

In this thesis, I have investigated stress physiology in relation to the regulatory mechanism behind morphological defence plasticity in a classic example of an anti-predator trait (**paper II-IV**). In addition, I have demonstrated that males express a more pronounced defence against predators than females and, further, that such sex-specific variation may be underlined by differences in gene expression levels of a vertebrate stress gene POMC (**paper V**). However, during my time as a PhD student, I was also fortunate to be able to examine how long-term differences in predation regime have influenced the evolution of sex-specific stress responses in a small-sized, live-bearing teleost prey species that inhabits many blue holes of Andros island, Bahamas. Below, I will briefly discuss that study and demonstrate that the findings are of interest for the general take home message of this thesis.

From the perspective of the model species, i.e. the Bahamas mosquitofish, *Gambusia hubbsi,* each blue hole is easily dichotomized into being either a low- or a high-risk environment, due to the presence/absence of one single piscivorous predator, i.e. the bigmouth sleeper, *Gobiomorus dormitor* (Langerhans et al. 2007; Heinen et al. 2013; Martin et al. 2014). This bimodal variation in predation risk has, since the colonization of the species ~15 000 years ago (Fairbanks 1989), resulted in repeated evolution of a suite of phenotypic traits (e.g. Langerhans, Gifford & Joseph 2007; Langerhans 2017; Riesch, Martin & Langerhans 2020). These evolutionarily isolated ecosystems are therefore well suited to employ as a natural experimental system for studying how predation risk has shaped the acute stress response in a vertebrate prey.

In total, we examined three populations from high predation sites and three populations from low predation sites. We used the opercular beat rate, i.e. ventilation frequency, as the dependent variable to compare the average stress response of

populations before and after an acute stressor. Ventilation frequency is a reliable proxy for stress, basically since the *fight or flight response per se* consists of a positive correlation of many physiological actions where increased ventilation rate is a key aspect of the response, and has therefore successfully been used in earlier stress studies on fish (see e.g. Hawkins, Armstrong & Magurran 2004; Brown, Gardner & Braithwaite 2005; Queiroz & Magurran 2005; Barreto *et al.* 2009; Di Poi *et al.* 2016).

We found sex-specific differences in ventilation frequency among wild-caught fish from high-risk populations. Specifically, females in high predation sites showed a lower ventilation frequency during an acute stressful situation as compared to male individuals (Figure 10). However, we found no evidence of sexual dimorphism in the acute stress response among populations from low predation sites. For sexually dimorphic species, such as the Bahamas mosquitofish where females bear live young and allocate more time towards foraging (Heinen et al. 2013), efficient energy utilization may be more important for females than for males. As discussed above, stress responses are costly, and, hence, organisms experiencing a high-risk environment with frequent stressful encounters need to prevent unwarranted energy expenditure by modulating the fright response. Further, we know that females from high predation environments have evolved larger brood sizes (Riesch, Martin & Langerhans 2013), and should, because of the relatively high investment into fecundity, pay a higher cost of energy expenditure into repeating fright responses from living in a world full of natural enemies. This may explain why the sexes differ in high-predation sites but not among the low-predation populations.

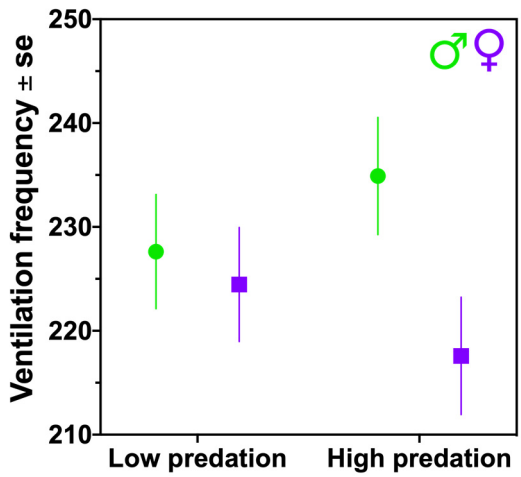


Figure 10. Average ventilation frequency in wild-caught Bahamas mosquitofish after an acute stress exposure. Males (circles) and females (squares) were derived from high- and low-predation sites (least-squares means ± 1 SE depicted).

In a broader context, these results indicate that the evolution of the acute physiological stress response differ between the sexes, and that this difference is molded by predation as the selective agent. This finding matches the result as I present in **paper IV**, where female crucian carp were found to express less morphological defence against predation. In addition, predator-exposure had no effect on POMC gene expression among females, whereas male differed so that one copy of POMC was upregulated in predator-exposed males as compared to non-exposed control males.

A methodological challenge

To make a long story short - a reader of this thesis does not need to be specifically perceptive to notice that it misses the obvious method to address the question if predation risk alters stress levels in crucian carp, and how this difference may be evident in the two dichotomous phenotypes. In teleost fish, cortisol is the main stress hormone, so, where are the cortisol measurements? In the bin. For some unknown reason it has been extremely difficult to get data of plasma cortisol concentration. I have spent a significant amount of time doing pilot studies, chasing fish, taking blood samples and centrifuging blood. I have analysed the plasma of stressed and non-stressed crucian carp, on crucian carp with implants containing cortisol, metyrapone or plain cocoa butter. I have done the analyses myself on ELISA (Enzyme-Linked-Immunosorbent-Assay) kits from several different manufactures, I have collaborated with the hospital in Malmö to run my samples using an ECLIA (Electro-Chemi-Luminescence-Immuno-Assay) method, and, further, I asked physiologists at Gothenburg University to run some samples by using a third method, RIA (Radio-Immuno-Assay). However, I repeatedly received very strange and non-logic data, with extreme variation within treatment groups and across methodology (see Figure 11). A clear source of error was the many negative values I got from fish across treatments – it is of course impossible to have negative values of a hormone. Hence, I had to move on with the project, leaving cortisol analyses behind and blame the failure on some sort of unknown matrix effect or demonic intrusion (Hurlbert 1984). Here, you might think that gene expression and the new technology for transcriptome sequencing (mRNA-seq) would be excellent methods to examine stress levels, and so much more, so let's have brief discussion about that.

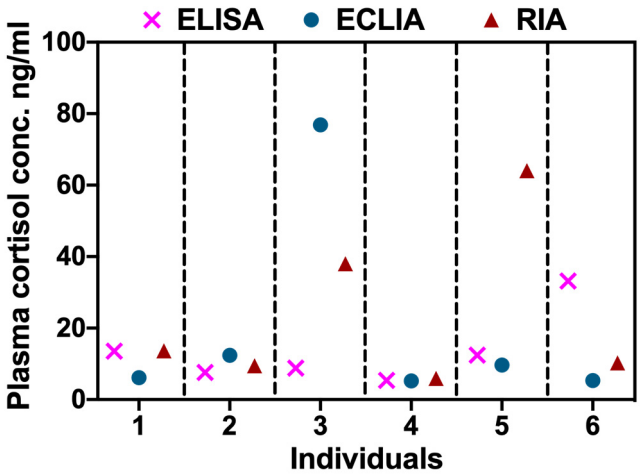


Figure 11. During my PhD project, I spent some significant amount of time trying to quantify cortisol levels in my experimental subjects. Because of some demonic intrusion, I never succeeded to get reliable results, despite testing multiple methods. One of the problems was an unknown cause of error in the methodology resulting in high intra-individual variation in cortisol concentration. This is clearly illustrated here, where individual 3, 5 and 6 show high degree of variation for the three different methods (ELISA, ECLIA and RIA) used to analyse cortisol concentrations.

A potential solution

Unravelling the molecular basis of phenotypic plasticity in morphological defence traits is a powerful step towards disentangling the proximate mechanism behind its regulation. Following exposure to manipulations of perceived risk (predator presence/absence) in a long-term experiment (eight months), I sampled organs (brain, kidney and muscle tissue) from totally 14 fish (7 predator-exposed and 7 non-exposed). From the organ tissue, we extracted the RNA that we subsequently forwarded for mRNA sequencing at SciLifeLab, Uppsala. This technique has opened a tremendous opportunity for me to identify regulatory gene networks responsible for the dramatic morphological transformation that crucian carps undergo when exposed to predation risk. Current questions that I am trying to address with this method are e.g. (i) what type of genes differ in expression between predator-exposed (deep-bodied) and non-exposed (shallow-bodied) crucian carp, (ii) how do they differ i.e. relative up-/down-regulation? Furthermore, I have employed this data set to examine the expression of POMC, i.e. the major stress gene among vertebrate species, see **paper IV** and **paper II** for more details.

Whole transcriptome analysis

Our de novo assembly resulted in a transcriptome holding high quality for further analyses of differential gene expression. In total, we obtained a transcriptome consisting of 112, 978 transcripts coding for 29, 683 genes). At the current stage, we do have some thrilling preliminary data, for example that the number of differently expressed genes between predator-exposed and non-exposed controls were 25 in total (Figure 12A). This number should be considered as few, in fact remarkably few from a whole genome perspective. However, the low number of differently expressed genes will absolutely simplify further work and facilitate a more proximate understanding of predator-induced plasticity. Following the findings of strong sexual dimorphism in both (i) defence expression and (ii) plasticity, as presented in **paper IV**, we have also analysed this data separated by sex. Here, it becomes even more intriguing. The number of genes that differed between predator-exposed and non-exposed control females were found to be dramatically higher than for the total sample (n tot = 119, see Figure 12B), and also compared to the male sample (Figure 12C). The most exciting result this far is probably the clear variation in the tissue-specific pattern when comparing the samples from females and males. The absolute majority of differential expression in female genes occur either in the brain or kidney, whereas male show their major difference in muscle tissue. From earlier research, we know that crucian carp increase their muscle mass (up to 20%) when co-existing with predators. This finding, in combination with the fact that the muscle tissue was sampled just anterior to the dorsal fin, i.e. within a region of the body were the defence is expressed, give support to the finding of sexual dimorphism in anti-predator strategies.

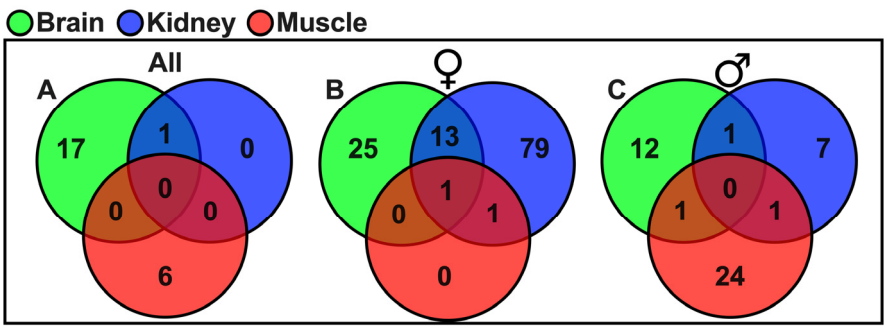


Figure 12. Illustration of the number of genes that were found to be differently expressed in brain, kidney and muscle tissue. Initially, statistical analyses were performed on the overall data set (A) including both males and females and testing for differences between predator-exposed and control. Subsequently, we divided the analyses following the findings of sex-specific differences in predator-induced plasticity (**paper IV**), and run the separate analysis for (B) predator-exposed females vs. control females and for (C) predator-exposed males vs. control males. Notice the intriguing difference between females (B) and males (C), where predation risk seems to influence female gene expression significantly with the brain and kidney tissue whereas males, who express relatively more morphological defence, show strong difference within muscle tissue sampled anterior of the dorsal fin i.e. where the morphological change is most evident.

Unpublished trait changes

Fin size plasticity

The dichotomous behaviour of crucian carp, directly controlled by the presence/absence of predators, is striking. A specific aspect that one notices almost immediately when observing the behaviour of experimental subjects under different predation risk is the contrasting use of the fins. Here, it is very clear that individuals, along with becoming more inactive and stationary, also expose their fins when being in close proximity of a predatory pike. Such behaviour may act in synergy with a deeper body depth and a darker body colouration to further enhance the signal to the enemy that I am a large, deep-bodied, hard-to-capture prey, which should be effective to prevent strike attempts by gape-size limited predators (Nilsson & Brönmark 2000). Such display of the fins would hence add to the adaptive value of a deeper body shape, and would, at least theoretically, be of more significance if it was followed by plastic enlargement of the fin areas; intriguingly – I found this to be the case.

In short, I exposed wild-caught and predator-naïve crucian carp to the presence/absence of a predatory pike for ca. six months. During the course of the experiment I recorded and subsequently quantified the behaviour of experimental subjects (n tot = 23, control = 11, predator-exposed = 12) as percentage of the total area of the dorsal fin that was expressed. In addition, I photographed all individuals when the experiment was terminated and extracted the total area of each fin. As illustrated in Figure 13, crucian carp have evolved a capacity to plastically increase the general size of the fins, a trait change that I suggest to be part of the integrated anti-predator phenotype. Moreover, an enlargement of fin area may enhance survival chances when a predator strikes, due to increased locomotor performance resulting in a higher acceleration speed and turning rate (Domenici *et al.* 2008).

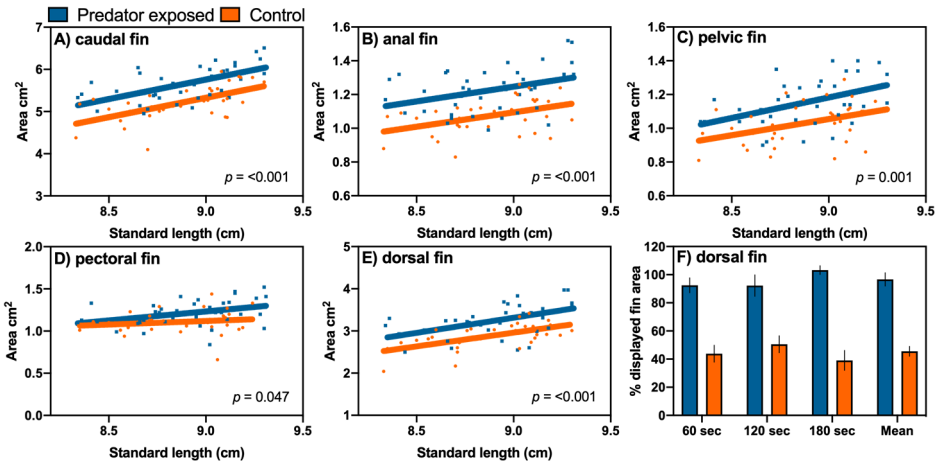


Figure 13. Novel trait plasticity: Fin size (A-E) enlargement in crucian carp as a response to predator-exposure. Individuals were either reared with a predatory pike (red) or without a predator (green) for ca. six months under controlled laboratory conditions. All fins are significantly larger in predator-exposed individuals. Interestingly, the pectoral fin, which is the only fin that is not differently expressed (personal observations) was found to be relatively less enlarged as compared to the other fins. The behaviour of displaying the fins when coexisting with natural predators was further quantified in a subsample of experimental subjects (n tot = 23), see Figure 13F. In short, individual fish were separated from the group and recorded within the experimental tank for 180 seconds. From these recordings, we calculated the displayed percentage of the total dorsal fin area a three different time points.

Brain-associated costs?

Among vertebrates, the brain is considered to require significantly more energy than other organs (Isler & van Schaik 2009). Hence, brain size of species and individuals (relative brain size) are often involved in strong resource allocation trade-offs, competing with the energetic demands of other phenotypic traits (Raichle & Gusnard 2002; Kotrschal, Kolm & Penn 2016). Recent studies have shown that predation is a major force in the evolution of brain size, where a "cognitive arms race" has been suggested to exist between predators and their prey. This hypothesis originates from a larger comparison of >600 pairs of predators and prey, demonstrating that the prey, on average, tend to have larger brains than their predators (Kondoh 2010). However, some studies have shown the opposite effect of brain size, i.e. that predation selects for smaller brain size (Samuk, Xue & Rennision 2018). However, despite numerous studies on how predators drive the evolution of brain size, few have examined predator-induced plasticity in brain morphology.

When I terminated the experiment included in **paper IV** and **V**, I took the opportunity to dissect out the brains from a subsample of experimental subjects (n = 44). My plan was to examine if non-lethal predation risk alters brain morphology, either in a positive direction where plasticity is adaptive and results in higher resource investment into

brain volume, or in a contradictive manner where predation risk results in stress-mediated costs or trade-off dynamics with e.g. morphological changes and, hence, less resources available for investments in brain development.

After some time in formaldehyde, for tissue fixation, all brains were photographed from the dorsal, lateral and ventral side. Subsequently, the volume of each brain region was calculated so that a comparison between the size of each brain structure could be compared between crucian carp exposed or not to a live predator for approximately six months. As illustrated in Figure 14, these preliminary results illustrate that the brain morphology does change following predator exposure. However, instead of adaptive plasticity I found evidence for a potential cost of non-lethal predation risk, basically since all analysed brain structures were found to be relatively smaller among individuals who were reared together with a live predatory pike as compared to the brains of control reared individuals (Figure 14). Hence, these preliminary result suggests that the high energetic demand from brain tissue is either reduced from stress-associated costs or traded off against other anti-predator traits. Or, *vice versa*, that predator-free laboratory conditions are so benign that the brain can undergo a plastic increase in size.

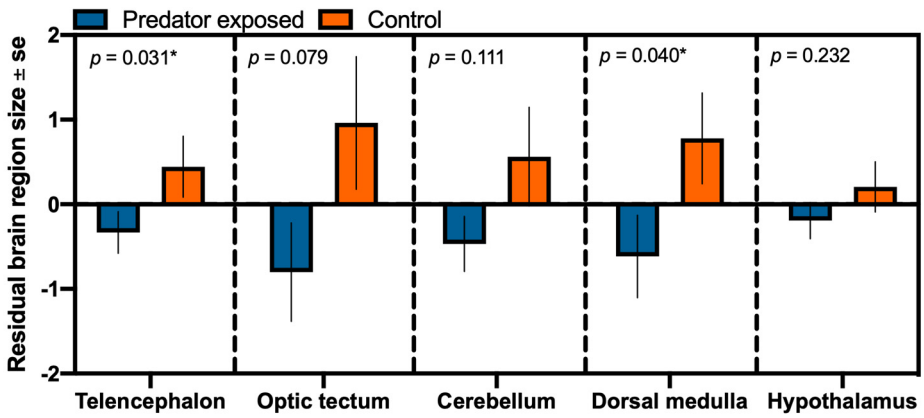


Figure 14. Preliminary data for predator-induced changes in brain morphology i.e. change in volume of specific brain regions. To highlight the main effects of treatment, residuals were calculated from regressing the raw region size (in mm^3) and total body weight (in g). Statistical values are obtained from five different GLM, one for each brain region. Treatment was used as fixed factor, brain region size as dependent variable and experimental tank nested within treatment. Body weight (g) and not standard length was used as a covariate with the argument that the correlation had a better fit and that crucian carp change growth direction from a horizonal to a vertical plane when exposed to predatory cues.

At the current stage, this is preliminary data and there is room for future improvements. For example, a lack of knowledge in brain anatomy led to an inadequate dissection methodology where I cut of the nervus tractus olfactorius i.e. the thin bilateral connection between the olfactory bulbs and the anterior part of the major structure (telencephalon). Hence, by mistake I left the olfactory bulbs in the crania that were

thrown in the organic waste, and by that, directly ruled out any possibility to examine the region of olfaction - a sensory system that should be of particular importance when living in a world of predators. Even though my knowledge in brain anatomy of cyprinid species have improved, it is still, despite formaldehyde fixation, quite tricky to dissect this small-sized organ. I should therefore remind myself and peers interesting in crucian carp brain anatomy to use the new technology of micro-computed tomography (micro-CT).

Acknowledgement

I would like to THANK all the funders that made my PhD-studies and this thesis possible.

The Swedish Research Council (VR)

Helge Ax:son Johnsons Foundation

Stiftelsen Jörgen Lindströms Stipendiefond

Lars Hiertas Foundation

The Royal Physiographic Society in Lund

Tack

Först och främst stort TACK **Christer**. Tack för att du trodde på mig och gav mig möjlighet att utforska projektets spännande frågor. Du är en förebild att se upp till - ordagrant med tanke på dina nästan 2 m. Du är en imponerande kunskapsbank med hjärtat på rätt plats. Tack för alla former av stöd under dessa år. Bättre handledning hade jag inte kunnat få.

Nästa stora TACK går till **Anders**. Precis som varenda doktorand knuten till vår enhet gjort de senaste 10 åren vill jag tacka dig för all teoretisk hjälp. Du är ett statistiskt unikum med ett autism-öga för detaljer. Tack för hjälp med statistik och grammatik, för biologi och ekologi. Tack också för alla filosofiska och för mig betydelsefulla diskussioner vi haft i Ekologihuset, Island, Nederländerna, USA och Portugal. Jag hoppas framtiden ger oss många fina samtal. ”Hur många?”

Det tredje och det näst sista stora TACKET går givetvis till dig **Kaj**. Du har varit en ypperlig guide i forskarvärlden sedan tiden för mitt master-projekt. Likt en Duracellkanin ångar du alltid på mot nya vetenskapliga upptäckter. Din drivkraft har betytt mycket för varje artikel i den här boken. Nu efter sisådär sju års vänskap kan jag tycka att det börjar bli dags att få följa med och testa gäddfisket på dina gamla ställen i ån.

Tack, all co-authors for great collaborations: **Brian Langerhans**, **Dan-E Nilsson**, **Helen Nilsson Sköld**, **Olof Berglund**, **Arne Hegemann**, **Jennie Örjes**, **Thomas Brodin**, **Jerker Fick**, **Bengt Hansson** and **Pallavi Chauhan**.

Brian, thank you one more time. Your knowledge and contribution to some of the papers in this thesis has absolutely improved the quality of my research.

Markus Lindberg, tack för din expertis och akuta insatser gällande genuttrycksanalyser. Tack för mer än ett decennium av fina stunder, intressanta samtal och lojal vänskap. Nu ser vi framemot din avhandling. Räknat i impact factor så kan den ju komma att gå till historien som den bästa.

Tack **Alexander Hegg**. Tack för alla diskussioner under de här åren. Tack för all support. Tack för Leonard Cohen. Du har lärt mig mycket om mycket och jag är otroligt tacksam för vår vänskap.

Kamelåså to you **Simon Herzog**! Kamelåså for being a great office neighbour. Kamelåså for always being a great friend. I hope we can go for a combined "daddy sparring session" with some fishing this summer.

Tack för att du räddade hela Bahamas-projektet **Gustaf**, jäkla hjälte! Tack för alla löpturer och samtal genom åren.

Tack för fina samtal genom åren **Emma J**. Jag uppskattar ditt sätt att se på världen.

Thank you **Marcus** for helping me to choose between "in" or "of" the very last minute.

Martin and **Raphael**, I don't know why, but you always remind me of the film Step brothers. Thanks for that ☺

Varpu, thank you for being a great office partner during this time.

To all PhD-students at the **Aquatic Ecology Unit** as I have come to know during these years, thank you for sharing this journey with me.

Tack för RNA-extraktionerna **Marie** och för att du alltid har ett leende på lager.

Emma Kritzberg, tack! Tack för ditt stöd. Tack för att du lyssnat på mitt religionsförakt under alla år. Tack för många intressanta och lärorika samtal om världens problem. Du är grym!

Lasse, tack för flera fina konferensminnen och för gott umgänge under dessa år.

Johan Hollander, tack för GENECO-tiden och för boktipset. Spot on!

Ben Chapman, thank you for the supervision during my master project. You and your alter ego Henrik are always fun, super smart and very kind.

Juan Pablo Almeida thanks for great friendship during our masters, and for our shared appreciation of Carl Sagan.

Tack till **Stefan Palm**, **Jonas Dahl**, **Johan Elmberg**, **Dubbel-Gunnar** och **Magnus Thelaus** för att ha inspirerat till fortsatta studier i Biologi.

Matilda Andersson, tack för att du hjälpt mig sortera döda fiskar och för de gånger vi spatserat genom Krankesjöns vassbälte till synes helt utan eftertanke och försiktighet. Hoppas vi lär oss elfiska med mer respekt framöver.

Stort tack **Svante Winberg** för all hjälp längs vägen, för att du tagit av din tid för att lära mig mer. Oavsett om det handlat om stress, serotonin, SSRI, melanin eller POMC så har du alltid gett långa utförliga och väldigt pedagogiska svar. Tusen Tack!

Dan Larhammar tack för din vänliga och hjälpsamma inställning och tack för POMC-diskussionerna.

Till **Danne, Erik** och **Matte** vill jag säga TACK för barndomens alla fiskeäventyr. Jamtland, Jamtland, jamt å ständut. Mäska sydväst!

Manfred, tack för lån av räv och tack för hjälp vid diverse fältarbeten men framförallt tack för mer än ett halvt liv av fin och lojal vänskap.

Från zoologi till botanik. Tack **Blomman**.

Tack **Dan** och **Kerstin** för att ni hejat på mig under mitt sista år som doktorand. Tack för att ni från första stunden välkomnat mig, Vidar och Sigrid med öppna armar. Ni är fantastiska!

Tack familjen **Kratz** och familjen **Åkerlund**. Jag förväntar mig inget annat än att detta blir nytt kursmaterial i biologi A och B på varenda gymnasieskola i Växjö, **Markus**.

Tack **mor**, tack **far**. Utifrån många spännande diskussioner har ni över tid tvingats inse att jag vägrar tro på existensen av fri vilja. Det är som sagt förmodligen en illusion. Mitt ödmjukaste tack går därför till er. Ni gav mig en någorlunda okej genuppsättning som i kombination med den miljö som ni erbjöd mig under barndomens första tid slutligen ledde fram till denna bok. Tack för nature och tack för nurtute. Tack för era oändligt stora hjärtan och för att ni alltid gett mig ert fulla stöd.

TACK **Vidar**, **Noelle**, **Sigrid** och **Otto** för att ni är så otroligt fina små underbara individer. Jag älskar att hänga med er. Nu när jag är biolog på riktigt vill jag ta tillfället i akt att lära er en viktig sak. Variation är en absolut förutsättning för evolution. Ju högre variation desto bättre. Ni tillsammans skapar därför ett gäng i absolut världsklass! Jag ser framemot att följa er under utvecklingen till adult stadium. Mot oändligheten och vidare!

Emma, det största TACKET går givetvis till dig. Ditt stöd under den här tiden har betytt långt mer än vad ord kan beskriva. Om man på något magiskt vis kan designa ett experiment med frågeställningen om du haft en positiv effekt på mig och då indirekt på den här avhandlingen så hade resultatet visat att vi kan förkasta H_0 med en statistik signifikans på rekordlåga: $p = 0.00000000000000000001$. **Emma**, bara med dig.

Till dig som jag av misstag glömt bort eller till dig som bara känner dig allmänt bortglömd, fyll i ditt namn här nedan:

Kära _____, tack för att du alltid _____ mig och för att du lärde mig _____. Hoppas innerligt vi får tillfälle att initiera något spännande samarbete i framtiden.

References

Ab Ghani, N.I., Herczeg, G. & Merilä, J. (2016). Effects of perceived predation risk and social environment on the development of three-spined stickleback (Gasterosteus aculeatus) morphology. *Biological Journal of the Linnean Society,* **118,** 520-535.

Abrams, P.A. (2000). The Evolution of Predator-Prey Interactions: Theory and Evidence. *Annual Review of Ecology and Systematics,* **31,** 79-105.

Agrawal, A.A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science,* **294,** 321-326.

Aguilera, G. (1994). Regulation of Pituitary ACTH-Secretion during Chronic Stress. *Frontiers in Neuroendocrinology,* **15,** 321-350.

Ahlgren, J., Yang, X., Hansson, L.A. & Brönmark, C. (2013). Camouflaged or tanned: plasticity in freshwater snail pigmentation. *Biology Letters,* **9,** 20130464.

Allen, M.J. & Sharma, S. (2020). Physiology, Adrenocorticotropic Hormone (ACTH). *StatPearls*. Treasure Island (FL).

Andersson, J., Johansson, F. & Soderlund, T. (2006). Interactions between predator- and diet-induced phenotypic changes in body shape of crucian carp. *Proceedings of the Royal Society B-Biological Sciences,* **273,** 431-437.

Bakus, G.J. (1981). Chemical defense-mechanisms on the great barrier-reef, Australia. *Science,* **211,** 497-499.

Banks, M.S., Sprague, W.W., Schmoll, J., Parnell, J.A.Q. & Love, G.D. (2015). Why do animal eyes have pupils of different shapes? *Science Advances,* **1**.

Barreto, R.E., Volpato, G.L., de Brito Faturi, C., Giaquinto, P.C., Gonçalves de Freitas, E. & Fernandes de Castilho, M. (2009). Aggressive behaviour traits predict physiological stress responses in Nile tilapia (Oreochromis niloticus). *Marine and Freshwater Behaviour and Physiology,* **42,** 109-118.

Bell, A.M. (2012). Animal behaviour: Personality in the wild. *Nature,* **491,** 341-342.

Bell, A.M., McGhee, K.E. & Stein, L. (2016). Effects of mothers' and fathers' experience with predation risk on the behavioral development of their offspring in threespined sticklebacks. *Current opinion in behavioral sciences,* **7,** 28-32.

Bengtson, S. (2002). Origins and Early Evolution of Predation. *The Paleontological Society Papers,* **8,** 289-318.

Bernier, N.J., Bedard, N. & Peter, R.E. (2004). Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. *General and Comparative Endocrinology,* **135,** 230-240.

Bernier, N.J. & Peter, R.E. (2001). Appetite-suppressing effects of urotensin I and corticotropin-releasing hormone in goldfish (Carassius auratus). *Neuroendocrinology,* **73,** 248-260.

Beston, S.M. & Walsh, M.R. (2019). Natural selection favours a larger eye in response to increased competition in natural populations of a vertebrate. *Functional Ecology,* **33,** 1321-1331.

Beston, S.M., Wostl, E. & Walsh, M.R. (2017). The evolution of vertebrate eye size across an environmental gradient: phenotype does not predict genotype in a Trinidadian killifish. *Evolution,* **71,** 2037-2049.

Boonstra, R. (2013). Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Functional Ecology,* **27,** 11-23.

Boonstra, R., Hik, D., Singleton, G.R. & Tinnikov, A. (1998). The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs,* **68,** 371-394.

Bradshaw, A.D. (1965). Evolutionary Significance of Phenotypic Plasticity in Plants. *Advances in Genetics* (eds E.W. Caspari & J.M. Thoday), pp. 115-155. Academic Press.

Brodie Jr, E.D. (1977). Hedgehogs use toad venom in their own defence. *Nature,* **268,** 627-628.

Brönmark, C. & Hansson, L.-A. (2012). *Chemical ecology in aquatic systems*. Oxford University Press, Oxford ; New York.

Brönmark, C. & Hansson, L.-A. (2012). *Chemical ecology in aquatic systems*. Oxford University Press, Oxford ; New York.

Brönmark, C., Lakowitz, T. & Hollander, J. (2011). Predator-Induced Morphological Plasticity Across Local Populations of a Freshwater Snail. *PLoS One,* **6,** e21773.

Brönmark, C. & Miner, J.G. (1992). Predator-induced phenotypical change in body morphology in crucian carp. *Science,* **258,** 1348-1350.

Brönmark, C. & Pettersson, L.B. (1994). Chemical cues from piscivores induce a change in morphology in crucian carp. *Oikos,* **70,** 396-402.

Brönmark, C. & Weisner, S.E.B. (1992). Indirect effects of fish community structure on submerged vegetation in shallow, eutrophic lakes: an alternative mechanism. pp. 293-301. Springer Netherlands, Dordrecht.

Brown, C., Gardner, C. & Braithwaite, V.A. (2005). Differential stress responses in fish from areas of high- and low-predation pressure. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology,* **175,** 305-312.

Brown, J.S., Laundré, J.W. & Gurung, M. (1999). The Ecology of Fear: Optimal Foraging, Game Theory, and Trophic Interactions. *Journal of Mammalogy,* **80,** 385-399.

Cal, L., Suarez-Bregua, P., Cerda-Reverter, J.M., Braasch, I. & Rotllant, J. (2017). Fish pigmentation and the melanocortin system. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* **211,** 26-33.

Cannon, W.B. (1915). *Bodily changes in pain, hunger, fear, and rage; an account of recent researches into the function of emotional excitement*. D. Appleton and Company, New York, London,.

Cannon, W.B. (1932). *The wisdom of the body*. W.W. Norton & Company, Inc., New York.

Caves, E.M., Sutton, T.T. & Johnsen, S. (2017). Visual acuity in ray-finned fishes correlates with eye size and habitat. *Journal of Experimental Biology,* **220,** 1586-1596.

Cerda-Reverter, J.M., Agulleiro, M.J., Guillot, R., Sanchez, E., Ceinos, R. & Rotllant, J. (2011). Fish melanocortin system. *European Journal of Pharmacology,* **660,** 53-60.

Chivers, D.P., Zhao, X.X., Brown, G.E., Marchant, T.A. & Ferrari, M.C.O. (2008). Predator-induced changes in morphology of a prey fish: the effects of food level and temporal frequency of predation risk. *Evolutionary Ecology,* **22,** 561-574.

Clinchy, M., Sheriff, M.J. & Zanette, L.Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology,* **27,** 56-65.

Cooke, S.J., Steinmetz, J., Degner, J.F., Grant, E.C. & Philipp, D.P. (2003). Metabolic fright responses of different-sized largemouth bass (Micropterus salmoides) to two avian predators show variations in nonlethal energetic costs. *Canadian Journal of Zoology,* **81,** 699-709.

Cott, H.B. (1940). *Adaptive coloration in animals*. Methuen & Co., London.

Creel, S. (2018). The control of risk hypothesis: reactive vs. proactive antipredator responses and stress-mediated vs. food-mediated costs of response. *Ecology Letters,* **21,** 947-956.

Creel, S., Christianson, D., Liley, S. & Winnie, J.A. (2007). Predation Risk Affects Reproductive Physiology and Demography of Elk. *Science,* **315,** 960.

Creel, S., Winnie, J.A. & Christianson, D. (2009). Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. *Proceedings of the National Academy of Sciences,* **106,** 12388.

Dahl, E., Orizaola, G., Winberg, S. & Laurila, A. (2012). Geographic variation in corticosterone response to chronic predator stress in tadpoles. *J Evol Biol,* **25,** 1066-1076.

Darwin, C. (1859). *On the origin of species by means of natural selection*. J. Murray, London,.

Dawkins, R. (1976). *The selfish gene*. Oxford University Press, New York.

Dhabhar, F.S. (2014). Effects of stress on immune function: the good, the bad, and the beautiful. *Immunol Res,* **58,** 193-210.

Di Poi, C., Lacasse, J., Rogers, S.M. & Aubin-Horth, N. (2016). Evolution of stress reactivity in stickleback. *Evolutionary Ecology Research,* **17,** 395-405.

Dodson, S. (1988). Cyclomorphosis in Daphnia galeata mendotae Birge and D. retrocurva Forbes as a predator-induced response. *Freshwater Biology,* **19,** 109-114.

Domenici, P., Turesson, H., Brodersen, J. & Brönmark, C. (2008). Predator-induced morphology enhances escape locomotion in crucian carp. *Proc. R. Soc. B,* **275,** 195-201.

Douglas, R.H. (2018). The pupillary light responses of animals; a review of their distribution, dynamics, mechanisms and functions. *Progress in Retinal and Eye Research,* **66,** 17-48.

Drouin, J., Trifiro, M.A., Plante, R.K., Nemer, M., Eriksson, P. & Wrange, O. (1989). Glucocorticoid receptor binding to a specific DNA sequence is required for hormone-dependent repression of pro-opiomelanocortin gene transcription. *Molecular and Cellular Biology,* **9,** 5305-5314.

Ducrest, A.-L., Keller, L. & Roulin, A. (2008). Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology & Evolution,* **23,** 502-510.

Eklöv, P. & Jonsson, P. (2007). Pike predators induce morphological changes in young perch and roach. *Journal of Fish Biology,* **70,** 155-164.

Ekström, C.U. (1838). Iakttagelser öfver formförändringenhos rudan (Cypr. Carassius Lin.). *Kongl. Vetenskaps-academiens Handlingar.*, 213-225.

Endler, J.A. (1978). A Predator's View of Animal Color Patterns. *Evolutionary Biology* (eds M.K. Hecht, W.C. Steere & B. Wallace), pp. 319-364. Springer US, Boston, MA.

Endler, J.A. (1991). Interactions between predators and prey. *Behavioral ecology: an evolutionary approach* (eds J.R. Krebs & N.B. Davies), pp. 169-196. Blackwell Scientific, Oxford.

Fairbanks, R.G. (1989). A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. *Nature,* **342,** 637-642.

Falk, R. (1995). The Struggle of Genetics for Independence. *Journal of the History of Biology,* **28,** 219-246.

Ferrari, M.C.O., Wisenden, B.D. & Chivers, D.P. (2010). Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology,* **88,** 698-724.

Fischer, C.P. & Romero, L.M. (2019). Chronic captivity stress in wild animals is highly species-specific. *Conservation physiology,* **7,** coz093-coz093.

Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity,* **115,** 276-284.

Fryer, J., Lederis, K. & Rivier, J. (1984). Cortisol inhibits the ACTH-releasing activity of urotensin I, CRF and sauvagine observed with superfused goldfish pituitary cells. *Peptides,* **5,** 925-930.

Fujii, R. (2000). The regulation of motile activity in fish chromatophores. *Pigment Cell Research,* **13,** 300-319.

Furtbauer, I., Pond, A., Heistermann, M. & King, A.J. (2015). Personality, plasticity and predation: linking endocrine and behavioural reaction norms in stickleback fish. *Functional Ecology,* **29,** 931-940.

Gaynor, K.M., Brown, J.S., Middleton, A.D., Power, M.E. & Brashares, J.S. (2019). Landscapes of Fear: Spatial Patterns of Risk Perception and Response. *Trends in Ecology & Evolution,* **34,** 355-368.

Giesing, E.R., Suski, C.D., Warner, R.E. & Bell, A.M. (2011). Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proc. R. Soc. B ,* **278,** 1753-1759.

Goldberg, A.D., Allis, C.D. & Bernstein, E. (2007). Epigenetics: A landscape takes shape. *Cell,* **128,** 635-638.

Gregory, T.R. & Wood, C.M. (1999). The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiol Biochem Zool,* **72,** 286-295.

Hammerschlag, N., Meyer, M., Seakamela, S.M., Kirkman, S., Fallows, C. & Creel, S. (2017). Physiological stress responses to natural variation in predation risk: evidence from white sharks and seals. *Ecology,* **98,** 3199-3210.

Harno, E., Ramamoorthy, T.G., Coll, A.P. & White, A. (2018). POMC: The Physiological Power of Hormone Processing. *Physiological Reviews,* **98,** 2381-2430.

Harris, R.M., Dijkstra, P.D. & Hofmann, H.A. (2014). Complex structural and regulatory evolution of the pro-opiomelanocortin gene family. *General and Comparative Endocrinology,* **195,** 107-115.

Hawkins, L.A., Armstrong, J.D. & Magurran, A.E. (2004). Predator-induced hyperventilation in wild and hatchery Atlantic salmon fry. *Journal of Fish Biology,* **65,** 88-100.

Hawlena, D. & Schmitz, Oswald J. (2010). Physiological Stress as a Fundamental Mechanism Linking Predation to Ecosystem Functioning. *The American Naturalist,* **176,** 537-556.

Hawryshyn, C.W. & Beauchamp, R. (1985). Ultraviolet Photosensitivity in Goldfish - an Independent Uv Retinal Mechanism. *Vision Research,* **25,** 11-20.

Heams, T., Huneman, P., Lecointre, G. & Silberstein, M. (2014). *Handbook of evolutionary thinking in the sciences*. Springer Berlin Heidelberg, New York, NY.

Hegemann, A., Matson, K.D., Versteegh, M.A., Villegas, A. & Tieleman, B.I. (2013). Immune response to an endotoxin challenge involves multiple immune parameters and is consistent among the annual-cycle stages of a free-living temperate zone bird. *J Exp Biol,* **216,** 2573-2580.

Hegg, A. (2020). *Adaptive and non-adaptive responses to toxin-producing cyanobacteria in water fleas*. Lund University, Lund.

Helfman, G.S. & Helfman, G.S. (2009). *The diversity of fishes : biology, evolution, and ecology,* 2nd edn. Blackwell, Chichester, UK ; Hoboken, NJ.

Hochberg, Z., Feil, R., Constancia, M., Fraga, M., Junien, C., Carel, J.C., Boileau, P., Le Bouc, Y., Deal, C.L., Lillycrop, K., Scharfmann, R., Sheppard, A., Skinner, M., Szyf, M., Waterland, R.A., Waxman, D.J., Whitelaw, E., Ong, K. & Albertsson-Wikland, K. (2011). Child Health, Developmental Plasticity, and Epigenetic Programming. *Endocr Rev,* **32,** 159-224.

Hodge, J.R., Alim, C., Bertrand, N.G., Lee, W., Price, S.A., Tran, B. & Wainwright, P.C. (2018). Ecology shapes the evolutionary trade-off between predator avoidance and defence in coral reef butterflyfishes. *Ecology Letters,* **21,** 1033-1042.

Höglund, E., Balm, P.H.M. & Winberg, S. (2000). Skin darkening, a potential social signal in subordinate Arctic charr (Salvelinus alpinus): The regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. *Journal of Experimental Biology,* **203,** 1711-1721.

Höglund, E., Weltzien, F.A., Schjolden, J., Winberg, S., Ursin, H. & Doving, K.B. (2005). Avoidance behavior and brain monoamines in fish. *Brain Research,* **1032,** 104-110.

Holling, C.S. (1965). The Functional Response of Predators to Prey Density and its Role in Mimicry and Population Regulation. *Memoirs of the Entomological Society of Canada,* **97,** 5-60.

Holopainen, I.J., Aho, J., Vornanen, M. & Huuskonen, H. (1997). Phenotypic plasticity and predator effects on morphology and physiology of crucian carp in nature and in the laboratory. *Journal of Fish Biology,* **50,** 781-798.

Holopainen, I.J., Tonn, W.M. & Paszkowski, C.A. (1997). Tales of two fish: The dichotomous biology of crucian carp (Carassius carassius (L)) in northern Europe. *Annales Zoologici Fennici,* **34,** 1-22.

Hossie, T.J., Ferland-Raymond, B., Burness, G. & Murray, D.L. (2010). Morphological and behavioural responses of frog tadpoles to perceived predation risk: A possible role for corticosterone mediation? *Ecoscience,* **17,** 100-108.

Hulthén, K., Chapman, B.B., Nilsson, P.A., Hollander, J. & Brönmark, C. (2014). Express yourself: bold individuals induce enhanced morphological defences. *Proc. R. Soc. B,* **281,** 20132703.

Hulthén, K., Chapman, B.B., Nilsson, P.A., Vinterstare, J., Hansson, L.A., Skov, C., Brodersen, J., Baktoft, H. & Brönmark, C. (2015). Escaping peril: perceived predation risk affects migratory propensity. *Biology Letters,* **11**.

Hurlbert, S.H. (1984). Pseudoreplication and the Design of Ecological Field Experiments. *Ecological Monographs,* **54,** 187-211.

Hyvärinen, H. (1985). Ecology and physiology of crucian carp [Carassius carassius (L.)] in small Finnish ponds with anoxic conditions in winter AU - Holopainen, I. J. *SIL Proceedings, 1922-2010,* **22,** 2566-2570.

Innes-Gold, A.A., Zuczek, N.Y. & Touchon, J.C. (2019). Right phenotype, wrong place: predator-induced plasticity is costly in a mismatched environment. *Proc Biol Sci,* **286,** 20192347.

Isler, K. & van Schaik, C.P. (2009). The Expensive Brain: A framework for explaining evolutionary changes in brain size. *Journal of Human Evolution,* **57,** 392-400.

Johannsen, W. (1911). The Genotype Conception of Heredity. *The American Naturalist,* **45,** 129-159.

Kittilsen, S., Schjolden, J., Beitnes-Johansen, I., Shaw, J.C., Pottinger, T.G., Sørensen, C., Braastad, B.O., Bakken, M. & Øverli, Ø. (2009). Melanin-based skin spots reflect stress responsiveness in salmonid fish. *Hormones and Behavior,* **56,** 292-298.

Kondoh, M. (2010). Linking learning adaptation to trophic interactions: a brain size-based approach. *Functional Ecology,* **24,** 35-43.

Koolhaas, J.M., de Boer, S.F., Coppens, C.M. & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology,* **31,** 307-321.

Kotrschal, A., Kolm, N. & Penn, D.J. (2016). Selection for brain size impairs innate, but not adaptive immune responses. *Proceedings of the Royal Society B-Biological Sciences,* **283**.

Kottelat, M. & Freyhof, J. (2007). *Handbook of European freshwater fishes*. Publications Kottelat.

Land, M.F. & Nilsson, D.-E. (2012). *Animal eyes,* 2nd edn. Oxford University Press, Oxford ; New York.

Landys, M.M., Ramenofsky, M. & Wingfield, J.C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *General and Comparative Endocrinology,* **148,** 132-149.

Langerhans, R.B. (2017). Predictability and Parallelism of Multitrait Adaptation. *Journal of Heredity,* **109,** 59-70.

Langerhans, R.B., Gifford, M.E. & Joseph, E.O. (2007). Ecological speciation in Gambusia fishes. *Evolution,* **61,** 2056-2074.

Lankford, S.E., Adams, T.E., Miller, R.A. & Cech, J.J., Jr. (2005). The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. *Physiol Biochem Zool,* **78,** 599-609.

Lastein, S., Hoglund, E., Mayer, I., Overli, O. & Doving, K.B. (2008). Female Crucian Carp, Carassius carassius, Lose Predator Avoidance Behavior when Getting Ready to Mate. *Journal of Chemical Ecology,* **34,** 1487-1491.

Leclercq, E., Taylor, J.F. & Migaud, H. (2010). Morphological skin colour changes in teleosts. *Fish and Fisheries,* **11,** 159-193.

Lees, A.D. & Waddington, C.H. (1942). The development of the bristles in normal and some mutant types of Drosophila melanogaster. *Proceedings of the Royal Society Series B-Biological Sciences,* **131,** 87-110.

Levis, N.A., Isdaner, A.J. & Pfennig, D.W. (2018). Morphological novelty emerges from pre-existing phenotypic plasticity. *Nature Ecology & Evolution,* **2,** 1289-1297.

Lima, S.L. & Dill, L.M. (1990). Behavioral decisions made under the risk of predation - A review and prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **68,** 619-640.

Maher, M.J., Werner, E.E. & Denver, R.J. (2013). Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proc Biol Sci,* **280,** 20123075.

Matson, K.D., Ricklefs, R.E. & Klasing, K.C. (2005). A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Dev Comp Immunol,* **29**.

McCollum, S.A. & Leimberger, J.D. (1997). Predator-induced morphological changes in an amphibian: Predation by dragonflies affects tadpole shape and color. *Oecologia,* **109,** 615-621.

McCollum, S.A. & Van Buskirk, J. (1996). Costs and Benefits of a Predator-Induced Polyphenism in the Gray Treefrog Hyla Chrysoscelis. *Evolution,* **50,** 583-593.

Metcalfe, N.B., Huntingford, F.A. & Thorpe, J.E. (1987). The influence of predation risk on the feeding motivation and foraging strategy of juvenile Atlantic salmon. *Animal Behaviour,* **35,** 901-911.

Meuthen, D., Baldauf, S.A., Bakker, T.C.M. & Thunken, T. (2018). Neglected Patterns of Variation in Phenotypic Plasticity: Age- and Sex-Specific Antipredator Plasticity in a Cichlid Fish. *American Naturalist,* **191,** 475-490.

Mitchell, M.D., Bairos-Novak, K.R. & Ferrari, M.C.O. (2017). Mechanisms underlying the control of responses to predator odours in aquatic prey. *Journal of Experimental Biology,* **220,** 1937-1946.

Murata, H., Shimada, N. & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J,* **168,** 28-40.

Nagano, M. & Doi, H. (2020). Ecological and evolutionary factors of intraspecific variation in inducible defenses: Insights gained from Daphnia experiments. *Ecology and Evolution,* **10,** 8554-8562.

Navarro, S., Soletto, L., Puchol, S., Rotllant, J., Soengas, J.L. & Cerda-Reverter, J.M. (2016). 60 YEARS OF POMC POMC: an evolutionary perspective. *Journal of Molecular Endocrinology,* **56,** 113-118.

Nilsson, D.E., Warrant, E. & Johnsen, S. (2014). Computational visual ecology in the pelagic realm. *Philos Trans R Soc Lond B Biol Sci,* **369,** 20130038.

Nilsson, D.E., Warrant, E.J., Johnsen, S., Hanlon, R. & Shashar, N. (2012). A Unique Advantage for Giant Eyes in Giant Squid. *Current Biology,* **22,** 683-688.

Nilsson, G.E. (1990). Long-term anoxia in crucian carp: changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. *Journal of Experimental Biology,* **150,** 295-320.

Nilsson, G.E. (2001). Surviving Anoxia With the Brain Turned On. *Physiology,* **16,** 217-221.

Nilsson, P.A. & Brönmark, C. (2000). Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. *Oikos,* **88,** 539-546.

Nilsson, P.A., Brönmark, C. & Pettersson, L.B. (1995). Benefits of a predator-induced morphology in crucian carp. *Oecologia,* **104,** 291-296.

Novoa, B., Bowman, T.V., Zon, L. & Figueras, A. (2009). LPS response and tolerance in the zebrafish (Danio rerio). *Fish Shellfish Immunol,* **26,** 326-331.

Oliveira, T.A., Koakoski, G., da Motta, A.C., Piato, A.L., Barreto, R.E., Volpato, G.L. & Barcellos, L.J.G. (2014). Death-associated odors induce stress in zebrafish. *Hormones and Behavior,* **65,** 340-344.

Paszkowski, C.A., Penttinen, O.P., Holopainen, I.J. & Tonn, W.M. (1996). Predation risk and feeding patterns of crucian carp. *Journal of Fish Biology,* **48,** 818-828.

Pettersson, L.B. & Brönmark, C. (1993). Trading off safety against food: state dependent habitat choice and foraging in crucian carp. *Oecologia,* **95,** 353-357.

Pettersson, L.B. & Brönmark, C. (1997). Density-dependent costs of an inducible morphological defense in Crucian carp. *Ecology,* **78,** 1805-1815.

Pettersson, L.B. & Brönmark, C. (1999). Energetic consequences of an inducible morphological defence in crucian carp. *Oecologia,* **121,** 12-18.

Pettersson, L.B. & Hedenström, A. (2000). Energetics, cost reduction and functional consequences of fish morphology. *Proceedings of the Royal Society B-Biological Sciences,* **267,** 759-764.

Pettersson, L.B., Nilsson, P.A. & Brönmark, C. (2000). Predator recognition and defence strategies in crucian carp, Carassius carassius. *Oikos,* **88,** 200-212.

Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. & Moczek, A.P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution,* **25,** 459-467.

Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology & Evolution,* **20,** 481-486.

Pontarotti, P. (2009). *Evolutionary biology : concept, modeling, and application*. Springer, Dordrecht Netherlands ; London ; New York.

Preisser, E.L., Bolnick, D.I. & Benard, M.E. (2005). Scared to Death? The Effects of Intimidation and Consumption in Predator-Prey Interactions. *Ecology,* **86,** 501-509.

Price, S.A., Friedman, S.T. & Wainwright, P.C. (2015). How predation shaped fish: the impact of fin spines on body form evolution across teleosts. *Proc. R. Soc. B,* **282**.

Quaye, I.K. (2008). Haptoglobin, inflammation and disease. *Trans R Soc Trop Med Hyg,* **102,** 735-742.

Queiroz, H. & Magurran, A.E. (2005). Safety in numbers? Shoaling behaviour of the Amazonian red-bellied piranha. *Biology Letters,* **1,** 155-157.

Raichle, M.E. & Gusnard, D.A. (2002). Appraising the brain's energy budget. *Proceedings of the National Academy of Sciences,* **99,** 10237-10239.

Ramsay, D.S. & Woods, S.C. (2014). Clarifying the roles of homeostasis and allostasis in physiological regulation. *Psychological review,* **121,** 225-247.

Relyea, R.A. (2003). Predators come and predators go: The reversibility of predator-induced traits. *Ecology,* **84,** 1840-1848.

Richter-Boix, A., Orizaola, G. & Laurila, A. (2014). Transgenerational phenotypic plasticity links breeding phenology with offspring life-history. *Ecology,* **95,** 2715-2722.

Riesch, R., Martin, R.A. & Langerhans, R.B. (2013). Predation's Role in Life-History Evolution of a Livebearing Fish and a Test of the Trexler-DeAngelis Model of Maternal Provisioning. *American Naturalist,* **181,** 78-93.

Riesch, R., Martin, R.A. & Langerhans, R.B. (2020). Multiple traits and multifarious environments: integrated divergence of morphology and life history. *Oikos,* **129,** 480-492.

Rohlf, F.J. (2017). TpsDig2. *Department of Ecology and Evolution, State Univ. New York, Stony Brook.*

Romero, L.M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology & Evolution,* **19,** 249-255.

Samuk, K., Xue, J. & Rennision, D.J. (2018). Exposure to predators does not lead to the evolution of larger brains in experimental populations of threespine stickleback. *Evolution,* 7**2,** 916-929.

Sapolsky, R.M. (1982). The endocrine stress-response and social status in the wild baboon. *Hormones and Behavior,* **16,** 279-292.

Sapolsky, R.M. (1990). Adrenocortical function, social rank, and personality among wild baboons. *Biological Psychiatry,* **28,** 862-878.

Sapolsky, R.M. (2004). *Why zebras don't get ulcers*. Third edition. New York : Owl Book/Henry Holt and Co., 2004.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev,* **21,** 55-89.

Schmitz, O.J., Beckerman, A.P. & O'Brien, K.M. (1997). Behaviorally Mediated Trophic Cascades: Effects of Predation Risk on Food Web Interactions. *Ecology,* **78,** 1388-1399.

Schneider, R.F., Li, Y., Meyer, A. & Gunter, H.M. (2014). Regulatory gene networks that shape the development of adaptive phenotypic plasticity in a cichlid fish. *Molecular Ecology,* **23,** 4511-4526.

Schwassmann, H.O. & Kruger, L. (1966). Experimental analysis of the visual system of the four-eyed fish Anableps microlepis. *Vision Research,* **5,** 269-281.

Schweitzer, C., Motreuil, S. & Dechaume-Moncharmont, F.X. (2015). Coloration reflects behavioural types in the convict cichlid, Amatitlania siquia. *Animal Behaviour,* **105,** 201-209.

Seiter, S.A. (2011). Predator presence suppresses immune function in a larval amphibian. *Evolutionary Ecology Research,* **13,** 283-293.

Selye, H. (1936). A Syndrome produced by Diverse Nocuous Agents. *Nature,* **138,** 32-32.

Selye, H. (1946). The General Adaptation Syndrome And The Diseases of Adaptation. *The Journal of Clinical Endocrinology & Metabolism,* **6,** 117-230.

Selye, H. (1973). The Evolution of the Stress Concept: The originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones. *American Scientist,* **61,** 692-699.

Sih, A., Bell, A. & Johnson, J.C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution,* **19,** 372-378.

Sinclair, A.R.E. & Arcese, P. (1995). Population Consequences of Predation-Sensitive Foraging: The Serengeti Wildebeest. *Ecology,* **76,** 882-891.

Sköld, H.N., Yngsell, D., Mubashishir, M. & Wallin, M. (2015). Hormonal regulation of colour change in eyes of a cryptic fish. *Biology Open,* **4,** 206-211.

Sköld-Chiriac, S., Nord, A., Nilsson, J.A. & Hasselquist, D. (2014). Physiological and behavioral responses to an acute-phase response in zebra finches: immediate and short-term effects. *Physiol Biochem Zool,* **87,** 288-298.

Skov, C., Brodersen, J., Brönmark, C., Hansson, L.A., Hertonsson, P. & Nilsson, P.A. (2005). Evaluation of PIT-tagging in cyprinids. *Journal of Fish Biology,* **67,** 1195-1201.

Skov, C. & Nilsson, P.A. (2018). *Biology and ecology of pike*. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Slominski, A., Wortsman, J., Luger, T., Paus, R. & Solomon, S. (2000). Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiological Reviews,* **80,** 979-1020.

Sommer, R.J., Dardiry, M., Lenuzzi, M., Namdeo, S., Renahan, T., Sieriebriennikov, B. & Werner, M.S. (2017). The genetics of phenotypic plasticity in nematode feeding structures. *Open Biology,* **7,** 160332.

Sterling, P. & Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. *Handbook of life stress, cognition, and health.* (eds S. Fisher & J. Reason), pp. 629-649. Wiley, Chichester, New York.

Stollewerk, A. (2010). The water flea Daphnia - a 'new' model system for ecology and evolution? *Journal of Biology,* **9,** 21.

Svanbäck, R. & Johansson, F. (2019). Predation selects for smaller eye size in a vertebrate: effects of environmental conditions and sex. *Proceedings of the Royal Society B: Biological Sciences,* **286,** 20182625.

Swain, P., Nayak, S.K., Nanda, P.K. & Dash, S. (2008). Biological effects of bacterial lipopolysaccharide (endotoxin) in fish: a review. *Fish Shellfish Immunol,* **25,** 191-201.

Tarkan, A.S., Almeida, D., Godard, M.J., Gaygusuz, O., Rylands, M., Sayer, C.D., Zieba, G. & Copp, G.H. (2016). A review and meta-analysis of growth and life-history traits of a declining European freshwater fish, crucian carp Carassius carassius. *Aquatic Conservation-Marine and Freshwater Ecosystems,* **26,** 212-224.

Taylor, S.E., Klein, L.C., Lewis, B.P., Gruenewald, T.L., Gurung, R.A. & Updegraff, J.A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol Rev,* **107,** 411-429.

Tollrian, R. (1995). Predator-Induced Morphological Defenses: Costs, Life History Shifts, and Maternal Effects in Daphnia Pulex. *Ecology,* **76,** 1691-1705.

Tollrian, R. & Harvell, C.D. (1999). *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton, N.J.

Tonn, W.M., Paszkowski, C.A. & Holopainen, I.J. (1989). Responses of Crucian carp populations to differential predation pressure in a manipulated pond. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* **67,** 2841-2849.

Uribe, C., Folch, H., Enriquez, R. & Moran, G. (2011). Innate and adaptive immunity in teleost fish: a review. *Veterinarni Medicina,* **56,** 486-503.

Välimaki, K., Herczeg, G. & Merila, J. (2012). Morphological anti-predator defences in the nine-spined stickleback: constitutive, induced or both? *Biological Journal of the Linnean Society,* **107,** 854-866.

Van Buskirk, J., McCollum, S.A. & Werner, E.E. (1997). Natural selection for environmentally induced phenotypes in tadpoles. *Evolution,* **51,** 1983-1992.

Veilleux, C.C. & Kirk, E.C. (2014). Visual Acuity in Mammals: Effects of Eye Size and Ecology. *Brain Behavior and Evolution,* **83,** 43-53.

Vinterstare, J., Hulthén, K., Nilsson, P.A., Nilsson Sköld, H. & Brönmark, C. (2020). Experimental manipulation of perceived predation risk and cortisol generates contrasting trait trajectories in plastic crucian carp. *The Journal of Experimental Biology,* **223,** jeb213611.

Waddington, C.H. (1942). The Epigenotype. *Endeavour,* **41,** 18-20.

Waddington, C.H. (1956). Genetic Assimilation of the Bithorax Phenotype. *Evolution,* **10,** 1-13.

Waddington, C.H. (1959). Canalization of Development and Genetic Assimilation of Acquired Characters. *Nature,* **183,** 1654-1655.

Weiss, L.C., Leese, F., Laforsch, C. & Tollrian, R. (2015). Dopamine is a key regulator in the signalling pathway underlying predator-induced defences in Daphnia. *Proceedings of the Royal Society B-Biological Sciences,* **282**.

West-Eberhard, M.J. (2003). *Developmental plasticity and evolution*. Oxford University Press, Oxford ; New York.

Winberg, S. & Thörnqvist, P.-O. (2016). Role of brain serotonin in modulating fish behavior. *Current Zoology,* **62,** 317-323.

Woltereck, R. (1909). Weitere experimentelle Untersuchungen uber Artveranderung, speziell uber das Wesen quantitativer Artunterschiede bei Daphniden. *Verhandlungen der Deutschen Zoologischen Gesellschaft Leipzig,* **19,** (110-173).

Ydenberg, R.C. & Dill, L.M. (1986). The Economics of Fleeing from Predators. *Advances in the Study of Behavior* (eds J.S. Rosenblatt, C. Beer, M.-C. Busnel & P.J.B. Slater), pp. 229-249. Academic Press.

Yin, M., Laforsch, C., Lohr, J.N. & Wolinska, J. (2011). Predator-induced defense makes Daphnia more vulnerable to parasites. *Evolution,* **65,** 1482-1488.

Young, K.V., Brodie Jr, E.D. & Brodie III, E.D. (2004). How the horned lizard got its horns. *Science,* **304,** 65-65.

Zanette, L.Y., White, A.F., Allen, M.C. & Clinchy, M. (2011). Perceived Predation Risk Reduces the Number of Offspring Songbirds Produce per Year. *Science,* **334,** 1398.

Zera, A.J. & Harshman, L.G. (2001). The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics,* **32,** 95-126.

This is water

Defence on Demand

I. Vinterstare, J, Hegemann, A, Nilsson, PA, Hulthén, K & Brönmark, C 2019, Defence versus defence: Are crucian carp trading off immune function against predator-induced morphology?, *Journal of Animal Ecology*, 88: 1510-1521.

II. Vinterstare, J, Hulthén, K, Nilsson, PA, Nilsson Sköld, H & Brönmark, C 2020, Experimental manipulation of perceived predation risk and cortisol generates contrasting trait trajectories in plastic crucian carp, *Journal of experimental biology*, vol. 223, jeb213611.

III. Vinterstare, J, Brönmark, C, Nilsson, PA, Langerhans, RB, Berglund, O, Örjes, J, Brodin, T, Fick, J, & Hulthén, K. Anti-predator phenotype in crucian carp altered by a psychoactive drug. *Submitted*.

IV. Vinterstare, J, Hulthén, K, Nilsson, PA, Langerhans, RB, Chauhan, P, Hansson, B, & Brönmark, C. Sex matters: different expressions of inducible morphological defence and stress genes between sexes in crucian carp. *Manuscript*.

V. Vinterstare, J, Hulthén, K, Nilsson, DE, Nilsson, PA & Brönmark, C 2020, More than meets the eye: Predator-induced pupil size plasticity in a teleost fish, *Journal of Animal Ecology*, 89:2258-2267.

LUND UNIVERSITY

Lund University
Faculty of Science
Department of Biology

ISBN 978-91-7895-755-2

Printed by Media-Tryck, Lund 2021 NORDIC SWAN ECOLABEL 3041 0903