Hormone strategies as a key for understanding life history trade-offs in fish

Camilla Håkonsrud Jensen

Thesis for the degree of Philosophiae Doctor (PhD)
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Camilla Håkonsrud Jensen

Summary

Animal behaviour has fascinated humans for millennia. For studying animal behaviour, evolutionary biologists have focused primarily on their ultimate fitness causes mainly using a top-down approach. In contrast, physiologists have concentrated on the proximate causes of behaviour adopting primarily a bottom-up approach. This difference in focus and methodology has caused a conceptual rift between the fields. To take part in narrowing this rift, this thesis has aimed to unite proximate mechanisms with ultimate evolutionary explanations. To reach this goal, we developed a digital modelling tool that describes the relationship between a simplified endocrine system and the behaviour of a generalised juvenile fish.

The model shows that the optimal growth hormones levels in juvenile fish decrease with size together with size-dependent mortality risk, while hormones that affect appetite and metabolism are kept relatively stable throughout the growth period. When comparing stable environments, we also found that optimal hormone levels increase with food availability.

In variable environments with partly predictable food availability, hormone levels increase when food availability is temporarily high, while they decrease when food availability is temporarily poor. In this way, we found that it is optimal for fish to:

- move their mortality costs over time by primarily foraging and growing when food availability is rich,
- build their energy reserves when food availability is at an intermediate level,
- and wait for the environment to improve when it is poor.

When a fish, in addition to living in a variable environment, is exposed to a parasite that only has an energetic cost to the host, its optimal compensation strategy is to increase its hormone levels and thus its growth, foraging and metabolism. As a result, fish also show increased predation mortality with increasing parasite costs. These are signs often associated with parasite manipulation, but the parasites in our model only take energy from the host and is devoid of any strategy.

In conclusion, we thus find that dynamic hormone levels have the potential to evolve as a unified strategy that affects survival and growth during the growth phase of juvenile fish. The model also indicates that such a hormone strategy can be adaptive to prepare the phenotype of a juvenile fish for the food availability and parasite costs that are likely to come in its environments. In addition we find that behavioural and physiological

changes following an infection might in some cases be the result of a co-evolved mixed phenotype of both the host and parasite, where both behave according to their own adaptive strategies. Using an approach including both proximate mechanisms and ultimate evolutionary explanations thus have the potential to increase our understanding of animal behaviour.

List of publications

Publications included in the thesis

PAPER I

Weidner, J., **Jensen, C.H.**, Giske, J., Eliassen, S. & Jørgensen, C. (in press) Hormones as adaptive control systems in juvenile fish. *Biology Open.* doi:10.1242/bio.046144

PAPER II

Jensen, C.H., Weidner, J., Giske, J., Budaev, S., Jørgensen, C. & Eliassen, S. (manuscript) Hormonal regulation of growth and survival in variable environments. Prepared for *OIKOS*.

PAPER III

Jensen, C.H., Weidner, J., Mennerat, A., Giske, J., Jørgensen, C. & Eliassen, S. (manuscript) Apparent manipulation: How parasites may modify their host's behaviour without using any tricks. Preprepared for *Ecology Letters.*

Contributions to other papers during the PhD period

Mennerat, A., Ugelvik, M.S., **Håkonsrud Jensen, C.**, Skorping, A., 2017. Invest more and die faster: The life history of a parasite on intensive farms. *Evolutionary Applications* 10, 890–896. doi:10.1111/eva.12488.

Weidner, J., **Jensen, C.H.**, Giske, J., Eliassen, S. & Jørgensen, C. (manuscript) Hormonal regulation of the phenotype into environmentally appropriate pace of life syndromes

Contents

1 Introduction

"Although reproduction is the overriding theme of the drama of life, feeding and being fed on, are the key subplots."

Lafferty and Kuris (2002)

1.1 Evolution and the phenotypic gambit

Evolutionary biology is, simply put, the study of why organisms look, function and behave the way they do. We often separate evolution into two categories where microevolution refers to the changes in gene frequencies from one generation to the next, while macroevolution refers to the alteration of organisms above the species level (e.g. the origin of new species). As proposed by Darwin (1859) in *On the Origin of Species* we have evolution by natural selection whenever: (1) Individuals within populations are variable, (2) some of this variability is heritable, (3) some individuals are more successful at surviving and reproducing than others and finally when (4) the successful individuals are not random but represent the individuals with the most favourable variation. These four postulates still hold true after being thoroughly tested over the years (see for example Freeman and Herron 2006), but now we usually refer to them in the language of genes.

Approximately 100 years after *On the Origin of Species* Tinbergen (1963) proposed that we study animal behaviour with four questions in mind. Specifically, he suggested that we focus on problems regarding their (1) development (i.e. ontogeny), (2) physiological causation, (3) survival value or function and (4) evolutionary history. The first two questions concern proximate physiological explanations, while the two remaining concentrate on ultimate evolutionary explanations.

To study the ultimate aspects of behaviour, evolutionary ecologists often employ a phenotypic approach, that ignore genetics and only look at the fitness of phenotypes (Kokko, 2007). By taking this view we assume that the basis of a behaviour or trait is controlled by the simplest genetic mechanisms possible. One example could be to assume a haploid genome where each distinctive behaviour is coded by a distinctive allele. Grafen (1984) criticised this purely phenotypic approach, renaming it the phenotypic gambit, as it can be misleadingly simple and, when taken literately, usually is false. Methods using a phenotypic approach can for example have problems dealing with heterozygous advantage (i.e. when heterozygotes have a higher fitness than homozygotes) and can produce unsatisfactory predictions and/or explanations when this is the case (Rubin, 2016). Still, as also pointed out by Grafen (1984), the phenotypic gambit has many advantages that makes it attractive, like saving time and being able to study traits where

we do not know the underlying genetics. However, it is only useful as long as we recognise that it is a simplification and "*provided that we remember that we may be wrong*" (Grafen 1984, p. 66).

In practice the phenotypic gambit often means that evolutionary ecologists not only ignore underlying genetics, but also other possible proximate mechanisms highlighted by the first two of Tinbergen's questions. These two questions have not been forgotten in biology, however, and are the main focus of the field of physiology. In contrast to evolutionary ecology, physiology often uses a more bottom-up approach to study behaviour, where it is the ultimate evolutionary explanations that ride in the back seat. This comes with its own set of problems, for example the potential for overlooking top-down effects and emerging properties in a system. Unfortunately, because of the differing methodological approach and focus of evolutionary ecology and physiology, there has arisen a communication barrier and a conceptual rift between the fields (Lessells, 2008). Recently, however, there have been calls from both sides for more holistic views to try to narrow this gap (Giske et al., 2013; Budaev et al., 2019; Lessells, 2008; Ricklefs and Wikelski, 2002; McNamara and Houston, 2009; Zera et al., 2007).

1.2 Hormones and growth in fish

The endocrine system is one of the physiological mechanisms often ignored when using a phenotypic approach. It is made up by glands secreting hormones that are transported via the bloodstream, and together with the nervous system makes up the body's main means of communication (Hiller-Sturmhöfel and Bartke, 1998). The endocrine system is better suited for a more widespread and longer lasting action in the body, in contrast to the rapid and more specific signalling of the nervous system. Hormones affect several aspects of the organism over different time scales; from relatively fast stress responses (Iwama, 2006) to longer lasting processes such as juvenile growth (Robson et al., 2002). Hormones also have the potential to mediate different trade-offs like the one between growth and survival (Sundström et al., 2004), and affect the probability that certain behaviours will happen (Squires, 2003). In this way the endocrine system represents one midpoint on the axis from genetics to behaviour.

Sexually reproducing fish have three main energy sinks; the basic maintenance of vital systems, somatic growth and sexual development (Barber et al., 2000), and the endocrine system affects all of them. Somatic growth is for example controlled by several hormone axes, with the major regulator being the growth hormones of the hypothalamic–pituitary–somatotropic axis (grey lines in **figure 1** p. 18; Mommsen, 2001). The thyroid hormones of the hypothalamic–pituitary–thyroid axis (black lines in **figure 1**) are

also very important for growth, development and metamorphosis in fish as they, among other things, affect the regulation of metabolic processes and energy use (Power et al., 2001). To supply the fish with energy for growth and metabolism, the "hunger hormone" ghrelin, the "satiety hormone" leptin as well as the orexin neuropeptides affect appetite and thus energy intake (Rønnestad et al., 2017). There are still more hormones that affect growth and appetite in fish, and this is further complicated by the highly interconnected nature and emerging properties of the endocrine system (Cowan et al., 2017).

Since hormone levels of individuals vary within populations, seem to be heritable (Fisher et al., 2007) and can affect survival, we should expect adaptive hormone levels to evolve in natural populations according to Darwin's postulates (see **section 1.1**). However, to survive, it is not sufficient that each individual hormone or hormonal axis works as a single unit alone. Even though we do not tend to think about it until it fails, an organism's physiology can be compared to an orchestra, where every single musician and instrument has to work as single units, as well as part of the whole. This can be easily illustrated if we imagine a scenario where a fish's growth and thyroid hormones are signalising increased growth and metabolism, while the hormones affecting appetite are signalling satiation. In a scenario like this the fish in question would certainly perish due to starvation. Hormones, then, should not only be expected to evolve as single units, but also as a combined hormone orchestra.

The environment, in addition to internal state of the organism, should influence which hormonal strategies could be considered advantageous and, therefore, which should be (at least temporarily) preserved in natural populations. Fish larvae with a high metabolism, are for example, found to be selected against in food-limited environments (Bochdansky et al., 2005). This has been proposed to weaken the selection pressure for higher growth rates in fish when resources are limited. In addition, domesticated brown trout (*Salmo trutta*) being bred for high growth in aquaculture facilities seem to experience increased predation mortality in the wild, due to reduced anti-predation behaviours (Johnsson et al., 1996). This effect was also found in wild brown trout injected with growth hormone. Increased predation mortality has also been reported in growth hormone transgenic coho salmon (*Oncorhynchus kisutch*, Sundström et al., 2004), Atlantic salmon (*Salmo salar*, Abrahams and Sutterlin, 1999) and channel catfish (*Ictalurus punctatus*, Dunham et al., 1999). We should, therefore, expect to see some interesting responses in the hormone strategies to the environment that fish are adapted to and grow up in, as we see with behaviour (Salvanes and Braithwaite, 2005; Salvanes, 2017). This is further indicated by the difference in timing of growth- and thyroid hormone expression found in different Atlantic salmon (*Salmo salar*) populations (Boeuf and le Bail, 1990).

1.3 Behavioural change following parasite infections

Parasites negatively affect the fitness of their hosts, the difference with predators being that they do not necessarily require the host to die, and typically attack only one victim per developmental stage (Lafferty and Kuris, 2002). Parasitic organisms represent a large fraction of the Earth's total biodiversity, and their lifestyle is one of the most widespread on our planet (Poulin, 2014). It should be unsurprising then, that fish in natural ecosystems are rarely found without parasites (Barber et al., 2000). In addition to basic maintenance, growth, and reproduction, parasites could, therefore, be considered a fourth energy sink for fish. Of these parasites some induce behavioural or physiological changes in their host following infection, and they are found to be very important in the maintenance of biodiversity and energy flow between habitats (Lefèvre et al., 2009b).

In the previous sections I have only considered situations where animals use their hormone strategies and resulting behaviours in ways that can be considered adaptive. However, Dawkins (1982) warns us that we should not always expect to see animals behave in ways that maximise their own fitness, but instead view host behaviour as the potentially fitness-maximising extended phenotype of a parasite. But in practice it can be very difficult to assess if the behaviour of an infected host is due to (1) host manipulation by the parasite, (2) host compensation or (3) a side-effect of the infection that is adaptive for neither the parasite nor the host (Poulin, 1995). This can be illustrated by hosts that forage more and thereby increase their exposure to predators following infection: In some cases this could be an adaptive response by hosts to compensate for increased energetic costs and reduced competitive ability (Milinski, 1990; Barber and Huntingford, 1995). In other cases it could reflect adaptive manipulation by trophically transmitted parasites, as it can increase the probability that they will get transferred to their next host (Hafer and Milinski, 2015, 2016; Hafer-Hahmann, 2019).

Because of the difficulty in assessing the ultimate cause of a change in an infected host by observation alone, physiological correlates following infection have been measured. As a result of this, some modifications have been found to be accompanied with changes in for example the endocrine- and/or nervous system of hosts (Klein, 2003; Escobedo et al., 2005). One example being that the serotonin levels of the gammarid *Gammarus lacustris* increase following infections by the parasite *Polymorphus paradoxus* (Maynard et al., 1996). Increased levels of this neuropeptide makes the host change its escape behaviour (Lefèvre et al., 2009b), indicating that it could be adaptive for the parasite as it can increase trophic transmission. However, as pointed out by Lefèvre et al. (2009b), the cause of these increased serotonin levels are still unknown.

In recent years there have been calls to change the focus of studies from correlation to

proximate causation, and to look for so-called manipulation factors of parasites (see for example Herbison et al. 2018; Thomas et al. 2005). In a few cases the cause of change is known, like for *Leucochloridium* spp. that invades the tentacles of their intermediate snail hosts making them pulsate and change colour (Wesołowska and Wesołowski, 2014). However, more often the causal link is still missing, and finding it has been equated by some to finding the smoking gun in a murder case (Poulin and Maure, 2015). The hope is that finding the causative manipulation factor in more parasite-host systems, will lead to an increased understanding of whether and how parasites might manipulate their host. Some of the proposed candidates so far are hormones, neurotransmitters, proteins, symbionts and interactions on a structural level (Herbison, 2017; Lafferty and Shaw, 2012).

1.4 Thesis aims

So far I have introduced relevant concepts to understand the works that make up this thesis. As a part of this I have indicated why knowing both the proximate and ultimate cause of behaviour is important, and some possible implications for focusing on only one of these aspects. In addition, I have highlighted the problem of knowing the cause of a behavioural change in a host following infection. And why increased insight in this field will depend not only on understanding the ultimate adaptive values for participants, but also the proximate causation of behavioural change.

This ties into the overarching goal of the Adapted Heuristics and Architecture" (AHA!) project (https://ahamodel.uib.no/), which is to *develop tools that mimic the proximate architecture for decision-making of an animal (often a fish) and thereby to understand what decisions it makes.* As part of reaching this goal, this thesis has aimed to:

1. Unite physiological proximate mechanisms with ultimate evolutionary explanations
2. Develop a digital modelling tool that describes the relationship between the hormone system and decision making in a growing juvenile fish
3. Apply this digital modelling tool to answer research questions

To aid in fulfilling these aims we also asked:

1. Do organisms have a unified hormone strategy to survive their growth periods?
2. What role does this potential combined hormone strategy play in preparing an organism's phenotype for future conditions?
3. Can studies of hormone strategies provide increased insight into the mechanisms of behavioural and physiological changes in hosts following parasite infections?

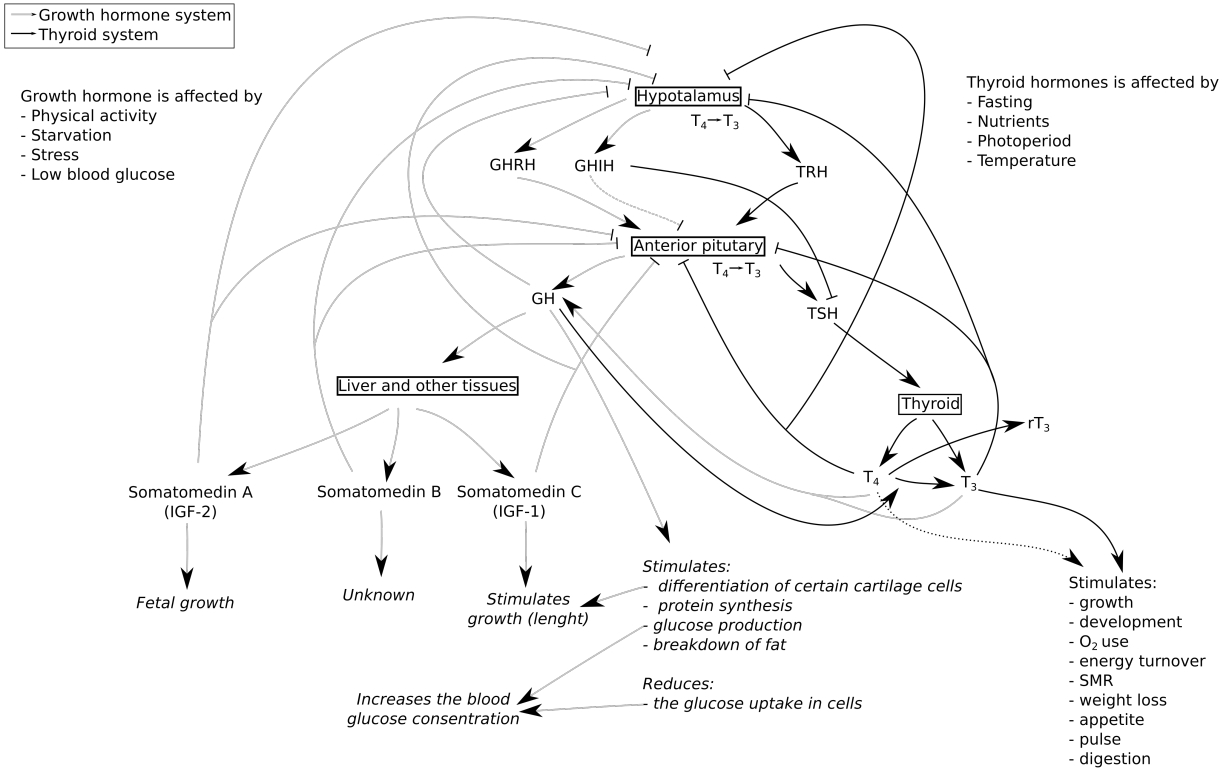

Figure 1: *A simplified illustration of the hypothalamic–pituitary–somatotropic axis (Growth hormone system) and hypothalamic–pituitary–thyroid axis (Thyroid system), how they affect growth and interact with each other. Drawn at a very early stage in our simplification process. Note for example that hormones affecting appetite are not part of this illustration. Pointed arrows indicate stimulation, flat arrows indicate inhibition while dotted lines indicate a weak effect. Abbreviations (please note that these hormones also are known under other names): Growth Hormone Releasing Hormone (GHRH), Growth Hormone Inhibiting Hormone (GHIH), Growth Hormone (GH), Insulin Growth Factor (IGF), Thyrotropin-Releasing Hormone (TRH), Thyroid Stimulating Hormone (TSH), triiodothyronine (T_3), reverse T_3(rT_3) and thyroxine (T_4). Illustration mainly based on Silbernagl and Despopoulos (1991), Robson et al. (2002) and Sand et al. (2006).*

2 Methods

"'That's the effect of living backwards,' the Queen said kindly: 'it always makes one a little giddy at first —'
'Living backwards!' Alice repeated in great astonishment. 'I never heard of such a thing!'
'— but there's one great advantage in it, that one's memory works both ways.'
'I'm sure mine only works one way,' Alice remarked. 'I can't remember things before they happen.'
'It's a poor sort of memory that only works backwards,' the Queen remarked."

Carroll (1871)

2.1 The hormone functions

The hormone system in the fish model that we have developed (**aim 2**) represents a very simplified version of the endocrine system, with its very diverse and interacting hormones. As we primarily wanted to focus on growth in the juvenile phase of fish, we narrowed the endocrine system down to the main hormones affecting growth, metabolism and appetite discussed in **section 1.2** (p. 14). We first started out with a concept similar to the one seen in **figure 1** (but note that hormones affecting appetite are not illustrated in this figure). However, a concept like this would be too convoluted and impossible to analyse with respect to cause and effect. A substantial amount of time was therefore needed to simplify the hormone system, using literature studies as our main method. Our model needed to be simple enough to be open for analysis, while complex enough to capture hormonal mechanisms.

At the end of the simplification process we were left with three hormone functions: The Growth Hormone Function (GHF), the Thyroid Hormone Function (THF) and the Orexin Function (OXF), that we refer to as "functions", to distinguish them from real molecules. **Paper I** documents this process and describes the different hormone axes that were simplified in our model in more detail. The resulting hormone functions each affect the physiology of the model fish in different ways (see **figure 2**) that I will summarise below. Please refer to **Paper I** for a full model description of the base model, **Paper II** for the implementation of an autocorrelated environment and **Paper III** for the implementation of a simple parasite.

The GHF level (γ [ng ml^{-1}]) affects energy allocation to growth ($\triangle W_{\text{structure}}$ [g week^{-1}]) in the following way:

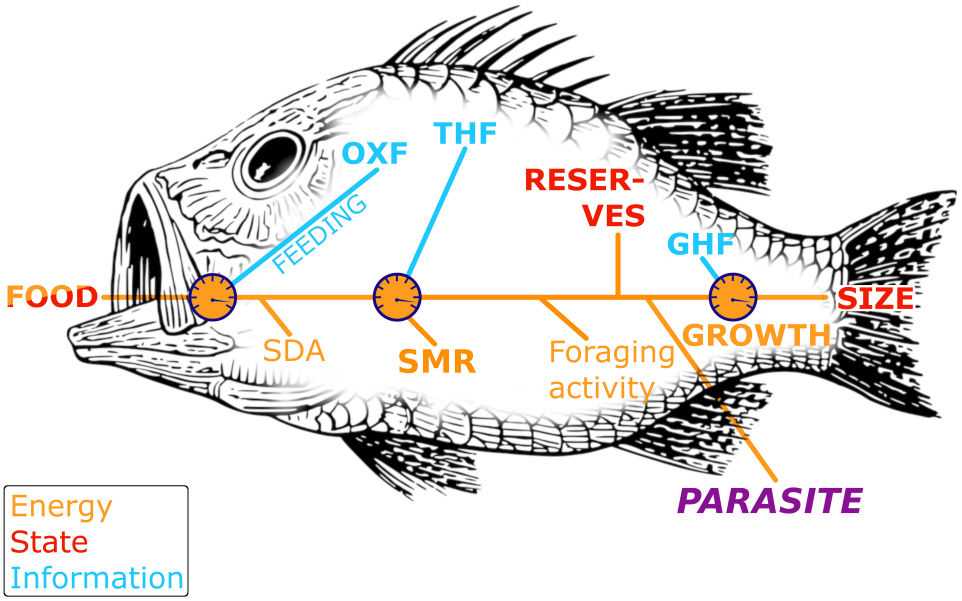

Figure 2: *The energetics, states and hormonal control of the hormone model (for a figure that includes the oxygen budget see* ***Paper I****). The food availability was introduced as an external state from* ***Paper II****, while the parasite was introduced in* ***Paper III****. Abbreviations: Specific dynamic action (SDA, energy needed to process food for use and storage) and standard metabolic rate (SMR). Modified from Figure 1 in* ***Paper I****.*

$$\triangle W_{\text{stucture}} = (\frac{\gamma}{\gamma_{\text{max}}}) \cdot k_{\text{growth}} \cdot W_{\text{structure}} \tag{1}$$

where γ_{max} is the maximum GHF level, k_{growth} is the maximum limit for proportional increase in the structural body mass in one time step [weeks] and $W_{\text{structure}}$ is the structural weight of the fish [g].

The effect of the THF level (τ [ng ml^{-1}]) is two-fold and regulates both the standard metabolic rate (SMR, P_{SMR} [J min^{-1}]) and the maximum possible oxygen uptake (A_{max} [J min^{-1}]):

$$P_{\text{SMR}} = [1 + (\frac{\tau}{\tau_{\text{max}}} - 0.5) \cdot k_{\text{THF\_SMR}}] \cdot P_{\text{standard}} \tag{2}$$

$$A_{\text{max}} = [1 + (\frac{\tau}{\tau_{\text{max}}} - 0.5) \cdot k_{\text{THF\_scope}}] \cdot A_{\text{standard}} \tag{3}$$

where τ_{max} is the maximum THF level, P_{standard} is the SMR based on total weight at $\frac{\tau_{\text{max}}}{2}$ [J min^{-1}], A_{standard} is the maximum oxygen uptake at $\frac{\tau_{\text{max}}}{2}$ [J min^{-1}], while

$k_{\text{THF\_SMR}}$ and $k_{\text{THF\_scope}}$ are the effects that THF has on P_{SMR} and $A_{\max}$, respectively.

The target intake (I [J min^{-1}]), most simply thought of as the appetite of the fish, is affected by the OXF level (α [pg ml^{-1}]):

$$I = \frac{\alpha}{\alpha_{\max}} \cdot k_{\text{OXF}} \cdot P_{\text{structure}} \tag{4}$$

where $\alpha_{\max}$ is the maximum OXF level, $P_{\text{structure}}$ is the SMR based on the structural weight of the fish [J min^{-1}] and is not affected by THF, and k_{OXF} is the effect that OXF has on intake.

The hormones also have consequences for the mortality of the fish: As the size-dependent mortality decreases with increasing size, the GHF level can influence the future instantaneous mortality and thus the survival of the fish throughout the growth period. By affecting the foraging activity, the OXF level influences the foraging and scope mortality components of the fish. This is because more time and energy need to be spent foraging under poorer food availability, at the cost of increased predator exposure (see **Paper II** for details). Finally, THF has a two-fold effect on mortality: On one side it increases the scope mortality through an increased metabolism, resulting in both higher energy- and oxygen demands. On the other side, THF also lowers this mortality component through an increased maximum oxygen use, thus raising the potential for escaping predators. All these mortality interactions make it possible to find the optimal levels of the hormone functions (i.e. the hormone strategy) that together best solve the trade-offs between growth and survival, resource availability and growth, as well as the one between foraging and avoiding predation.

2.2 Dynamic optimisation modelling

Models are often criticised for being too simple (see for example Birks 1997 and Chu et al. 2003), but in my personal experience they are also sometimes criticised by reviewers for being too complex. Sometimes this criticism is based on real concerns, as when unvalidated models are used to answer questions regarding high stake problems like global climate change (Gross and Strand, 2000). But often this criticism seems to be rooted, at least in part, in the same discussions that surrounds reductionism. There also seems to be a misconception that models are supposed to perfectly represent nature. However, models are not a way of investigating nature, instead they are used to investigate our own thinking and the logic of our arguments (Kokko, 2007). Therefore, models are very useful for answering "what-if" questions and this makes them a valuable tool for developing theory and our understanding of evolutionary problems. As nature is rather complex,

simplification is often needed to gain a better understanding of the whole, in the same way that a simplified map is better for navigation than a hypothetical 1:1 map. This is where modelling turns into an art form; with the application in mind, the modeller has to figure out which aspects that are necessary and which can be simplified or excluded. Still models shine their brightest when combined with empirical experiments and observations, as this makes testing and refinement possible, while at the same time helping to stimulate new ideas and hypotheses (Grimm and Railsback, 2005).

The main method for this thesis has been dynamic state-variable optimisation modelling (Clark and Mangel, 2000). In evolutionary optimality modelling the underlying

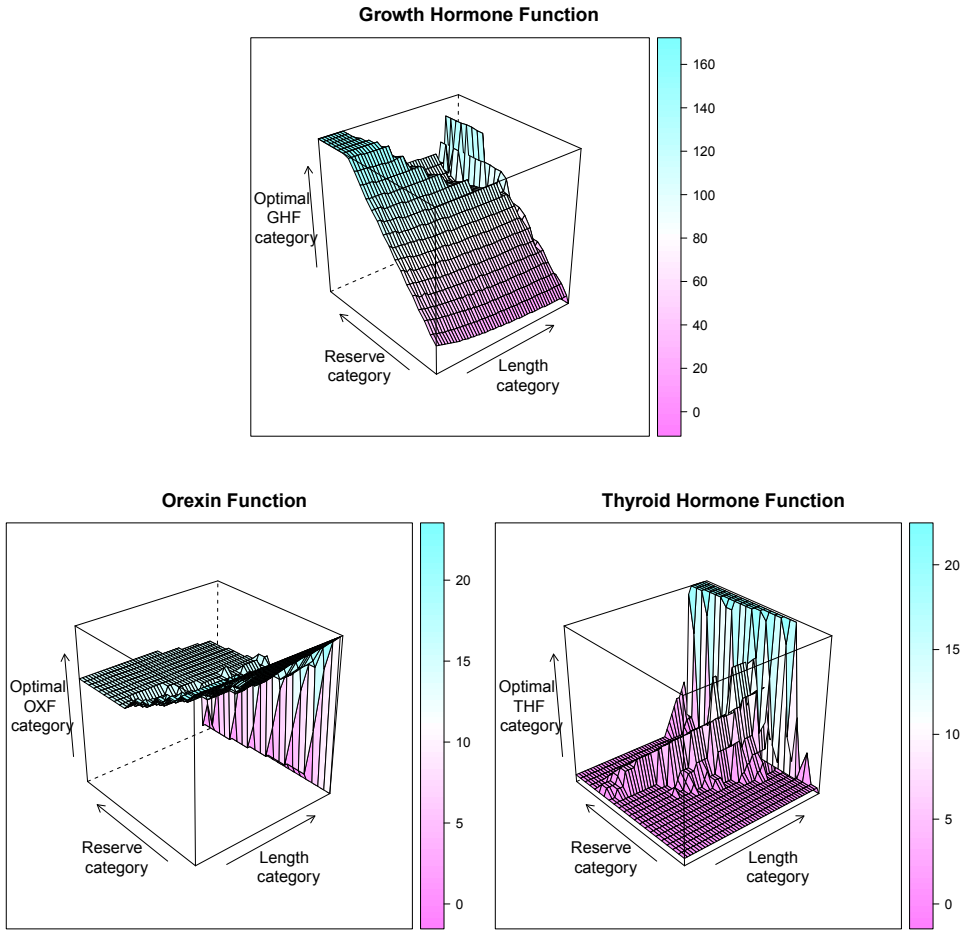

Figure 3: *A graphical representation of a lookup table produced by the backwards procedure of the hormone model (using* ***Paper II*** *parameters) in a stable environment with average food availability.*

assumption is that there exists an evolutionarily optimal phenotype in every situation, and that we therefore can ignore underlying genetic mechanisms (i.e. use a phenotypic approach, see **section 1.1** p. 13). This can be very useful as we give the model the possibility to find optimal solutions without the limitations of potentially incorrect genetic assumptions. Because of this, optimality models are very good for studying which (optimal) strategies should evolve under certain conditions (Pigliucci, 2005).

As dynamic optimisation models are used to study fitness maximisation under a set of limitations (i.e. trade-offs) the proxy for fitness used by the researcher is important; it can for example be an equal fitness reward for all tactics that result in surviving a growth period (like in our model), or a diminishing reward based on which tactics finish first or within a set time. This is important because it is not the fitness proxy in every time step that is optimised, but the strategy that yields the highest combined fitness for the whole growth period. As a result, the best solution in every time step depends on the current state, and not how one got into this state. Because of this, the optimal strategy in a dynamic state-variable optimisation model is calculated in a backwards iterative process that moves from the last to the first time step. Knowing where you want to end up makes planning easier, or in this case finding the optimal strategy. After the backwards iteration one is left with a lookup table that shows the optimal strategy depending on each state, similar to the one in **figure 3**. Many optimisation models stop at this step, but some, like ours, then go on to do a forward simulations of individuals that behave according to the optimal strategy.

Like any other modelling type, or method in general, optimisation models are not without their drawbacks. Real organisms are not all-knowing computers that are able to do super complex mathematical calculations at a moment's notice, but will instead often behave suboptimally probably according to rules of thumb (Budaev et al., 2019; Giske et al., 2014; Eliassen et al., 2016). The phenotypic approach used in optimality models also make them vulnerable to producing optimal strategies that cannot evolve in natural populations, either because of underlying phenotypic- or genetic mechanisms that limits the evolution of this trait (see also **section 1.1** p. 13). For example, is there enough genetic variability in the population to produce this strategy, or is it too good to be true? Optimality models can therefore never be used to answer if a species will display a certain behaviour, but it can, however, be used to study which types of behaviours would be advantageous if we assume that the genetic machinery produces the most optimal phenotype (Kokko, 2007). As with other modelling approaches, one also runs into the possibility of modelling fantasy worlds if not being careful, and it is therefore important to always make models that are testable through empirical studies either now or in the future.

As our goal is to investigate how hormones potentially can evolve in adaptive ways to regulate growth and survival in animals living in their environments, optimality models are a good tool, as they help us to investigate if this is possible in theory. Even if the model is a simplification, it is also able to highlight what hormone strategies can evolve in natural populations, which helps us meet our **1st aim** (see **section 1.4** p. 17) of combining proximate mechanisms with ultimate explanations. By validating our model against empirical observations (see **Paper I**) we can also make sure that we are at least on the right track. However, as measuring hormones in fish at this point in time can be a tricky procedure, it is probably not testable at the moment, but hopefully will be in the future.

2.3 Model differences across the papers

The work presented in **Paper I** represents the original base model, and the paper can be seen as our model description that the other papers build upon. In this version of model the environment had a stable food availability that did not change during the growth period of the individual fish. As there was no source of stochasticity in this model, each simulation represented one individual fish.

In **Paper II** we extended the model in **Paper I** by including a stochastic food availability. This inclusion also meant that we now were modelling a population of fish, as each individual fish experienced a different temporal trajectory in terms of food availability, and thus has differing state and hormone tactics at each point in time. The practical implications of this is that some of the parameter values used in **Paper I** needed to be re-parametrised to fit this new scenario (see **table 1** for the parameters that were changed). Please note that this did not affect the resulting patterns in the hormone functions.

Because of this re-parametrisation, the constant environment presented in **Paper I** was no longer directly comparable to the variable environment in **Paper II**. When comparing the hormone adaptations in constant and variable environments in **Paper II**, we therefore chose to run new simulations with the new parameter values for the constant environments. As a result, the constant environments presented in **Paper I** differ slightly from those in **Paper II.**

In **Paper III** we extended upon the model from **Paper II** with a simple parasite that only takes energy from its host in a way that scales with the structural weight of the fish. By doing this we make no assumption about the life-history of the parasite, or the number of parasites infecting the host. We also introduce a starvation level, so that survival approaches zero when reserves falls below a set threshold ($k_{\text{starvation}}$). In simulations using this model version we use the same parameter values as those introduced

in **Paper II** (except for $E_{\max}$), with the addition of the new parameters introduced in this version (see **table 1**).

Table 1: *The parameter values that changed across the papers. Empty values indicate that the parameter was not in use or did not exist. For a full list of parameters and variables and their symbols please see the respective papers.*

Symbol	Paper I	Paper II	Paper III	Unit	Definition
E	0.9-1.1	0.36-1.64			Food availability in constant environments
$E_{\max}$		1.64	1.40		Maximum food availability
$E_{\min}$		0.36	0.36		Minimum food availability
$E_{\text{E\_autocorr}}$		0.8	0.8		Autocorrelation constant for the food availability
$E_{\text{E\_sd}}$		0.35	0.35		The number of standard deviations that corresponds to $E_{\min}$ and $E_{\max}$
k_{OXF}	5	8.5	8.5		The effect OXF has on intake (I)
k_{parasite}			0.0-1.0		The parasite exploitation level
$k_{\text{starvation}}$			0.01		The starvation level of the fish. When reserves fall below this proportion, the survival approaches 0.
$k_{\text{THF\_scope}}$	0.24	0.2	0.2		Effect of THF on A_{standard}(maximum possible oxygen uptake at $\tau_{\max}/2$)
$k_{\text{THF\_SMR}}$	0.23	0.25	0.25		Effect of THF on P_{standard}(SMR at $\tau_{\max}/2$ based on total weight W)
m_{fixed}	0.01	0.01	0.01	year^{-1}	Size-independent mortality
m_{foraging}	0.08	0.03	0.03	year^{-1}	Foraging mortality coefficient
$m_{\text{foraging}\times\text{scope}}$	0.9	1.2	1.2	year^{-1}	Active-while-vulnerable mortality coefficient
m_{scope}	0.8	1.3	1.3	year^{-1}	Scope mortality coefficient
m_{size}	0.038	1.3	1.3	year^{-1}	Size-dependent mortality coefficient
x_{foraging}	2	3	3		Foraging mortality exponent
x_{scope}	3	2.7	2.7		Scope mortality exponent
x_{size}	-0.75	-0.75	-0.75		Size-dependent mortality exponent
$\alpha_{\max}$	1500	2500	2500	pg ml^{-1}	Maximum OXF level
$\gamma_{\max}$	200	200	200	ng ml^{-1}	Maximum GHF level
$\tau_{\max}$	5	5	5	ng ml^{-1}	Maximum THF level

3 Results

> *"Far better an approximate answer to the* right *question, which is often vague, than an* exact *answer to the wrong question, which can always be made precise."*
>
> Tukey (1962)

In this section I will not summarise the papers that make up this thesis, for that I refer to the abstract of each respective paper. I will, however, try to answer the questions asked in **section 1.4** (p. 17) based on the results from the papers.

All papers presented in this thesis aim to narrow the conceptual rift that often has separated the fields of physiology and evolutionary ecology. First by illustrating the development (**Paper I** & **II**) and then the usage (**Paper III**) of an optimisation model that combines proximate physiological mechanisms with ultimate evolutionary explanations (**aim 1**).

Paper I represents our work to arrive at a model of hormonal control for feeding, growth and survival in growing juvenile fish. To achieve this, we simplified the immense complexity of the endocrine system into three aggregated hormone functions which we named the Growth Hormone Function (GHF), the Thyroid Hormone Function (THF) and the Orexin Function (OXF) (see **section 2.1** p. 19). In this paper we also presented the optimisation model based on this much simplified endocrine system for the first time, and verified it against the literature by optimising the behaviour of growing generalised juvenile fish in environments with constant food availability. In **Paper II** we extended the model presented in **Paper I** by introducing uncertainty into the environment by making food availability vary over time. These two papers together represent our work towards developing a digital modelling tool that describes the relationship between the hormone system and decision-making in a growing juvenile fish (**aim 2**).

In **Paper III** we extended the model even further by adding a simple parasite that only takes energy from its host. We then used this extended model as a tool to answer a research question (**aim 3**), specifically if a simple parasite that only takes energy from its host can lead to something that looks like "advanced" multidimensional parasite manipulation.

3.1 Optimising the dynamic hormone levels as a strategy to survive the growth period

In **Paper I** we followed a growing juvenile fish in a constant food environment (i.e. constant food availability). In this environment we saw that it was optimal that the orexin hormone function (OXF) and the thyroid hormone function (THF) were kept relatively

stable throughout the growth period. There was some variation in THF, however, but not enough to have a visible effect on either the standard metabolic rate (SMR) or the maximum oxygen uptake of the model fish. However, due to gains in body mass, the SMR and oxygen uptake still increased throughout the growth phase. The fish's length increased in a relatively linear fashion, which was achieved by optimal growth hormone function (GHF) levels declining during the growth phase. The instantaneous mortality rate also decreased, most of all in its size-dependent component.

We also compared the hormone strategies of fish in different constant food environments in **Paper I**, and found that the optimal hormone function levels increased with the food availability across these environments. This resulted in higher growth rate, food intake, SMR and survival during the growth phase.

Notably, a correlation between the hormone functions emerged from the model in **Paper I**: In richer (but still constant) environments, it was optimal to grow faster and invest more in growth, which was driven by increased GHF levels. This faster growth demanded a higher need for energy, so OXF was also increased in richer environments. THF then helped to cover the energy needed by increasing the metabolism and decreasing the scope mortality through a raised maximum oxygen uptake. Both the thyroid and growth hormone axes have been described to have an effect on growth (see for example Robson et al. 2002; Chang et al. 2012; Mommsen 2001), which makes this correlation between GHF and THF especially interesting, as THF has no direct effect on growth in our model.

In **Paper II** we also looked at some stable environmental scenarios (mainly as a comparison with a variable environment). As a result of the re-parametrisation with the extension to a variable environment (see **section 2.3** p. 24) these scenarios differed slightly from those in **Paper I**: The main difference being that fish increased their OXF levels in the environment with the poorest food availability to avoid starvation.

As we found that the model was able to find realistic hormone strategies that led to reasonable growth and survival in constant environments in **Paper I**, we could expand our research ambition to also investigate how fish may use their hormone strategy to cope with temporally varying environments. In **Paper II** we therefore replaced the constant food environment with food availability that varies in an autocorrelated manner through time. Due to this model change we could now also look at a population of fish in an environment, with each individual experiencing different food availabilities over time, which caused individual variation in physiological states and hormone tactics. We found that fish that on average experienced high food availability had higher optimal hormone function levels, while the opposite was true for fish that on average experienced poor food availability. And similar to the results in **Paper I**, it was optimal for lucky fish that mainly experienced rich food availability to have higher growth rates, intake, SMR

and as a result a greater survival at the end of the growth phase. However, we also saw that the consequences of living according to the optimal policy was that these individuals experienced a much higher mean instantaneous mortality risk throughout their growth period, than their slower growing counterparts that made optimal decisions under poorer food availability.

In general, we saw that it was optimal for model fish to increase their hormone function levels when the food availability was high in **Paper II**. We interpret this as a short-term optimal tactic to take advantage of the temporarily increased resource availability. Similarly, it was optimal for fish to decrease their hormone function levels when the food availability was poor, which we interpret as a means to survive until the environment improves again. This shift in optimal hormone function usage with environmental conditions, resulted in high growth, foraging, metabolism and thus increased scope- and foraging mortality when the food availability was rich, while the opposite was true when it was poor. For some unlucky individuals, however, that had experienced poor food availability over a very long time, it eventually became optimal to switch to a desperate hormone tactic by raising their THF and OXF levels even though the environment remained poor. The result of this was an increased foraging activity and metabolism to avoid immediate death due to starvation. The optimal policy of THF both when the food availability was high and poor made it obvious that the fish in **Paper II,** not only used THF to support growth when the environment was rich, but also to lower their scope mortality when foraging by elevating their maximum possible oxygen uptake.

In **Paper III** we further added a parasite that only took energy from the model fish. Even though the main goal of this paper was to investigate the mechanisms of behavioural changes in hosts following parasite infections, the paper also gave us insight into the optimal hormone strategies under parasite infections. In general, we saw that with increasing parasite exploitation levels (i.e. increased energetic costs of harbouring parasites), the optimal hormone function levels also increased, and thus fish could switch to a strategy with potentially faster growth (see **section 3.3** p. 32). However, even if not discussed in **Paper III**, we also saw that individuals optimised to different parasite exploitation levels varied a bit in their hormone strategies depending on the food availability (**figure 4**): Fish optimised for all levels of parasitism elevated their OXF levels as the environment improved (**figure 4b**), as also seen in **Paper I** and **II**. More interestingly, fish optimised towards higher parasite exploitation raised their THF levels under poorer food availabilities than individuals that were optimised towards small or no parasite costs (**figure 4c**). This counteracted an increase in scope mortality due to higher OXF levels, thus lowering overall instantaneous mortality. In other words, we saw that these two hormone functions were optimised to mitigate each other, which would be unnoticeable if we

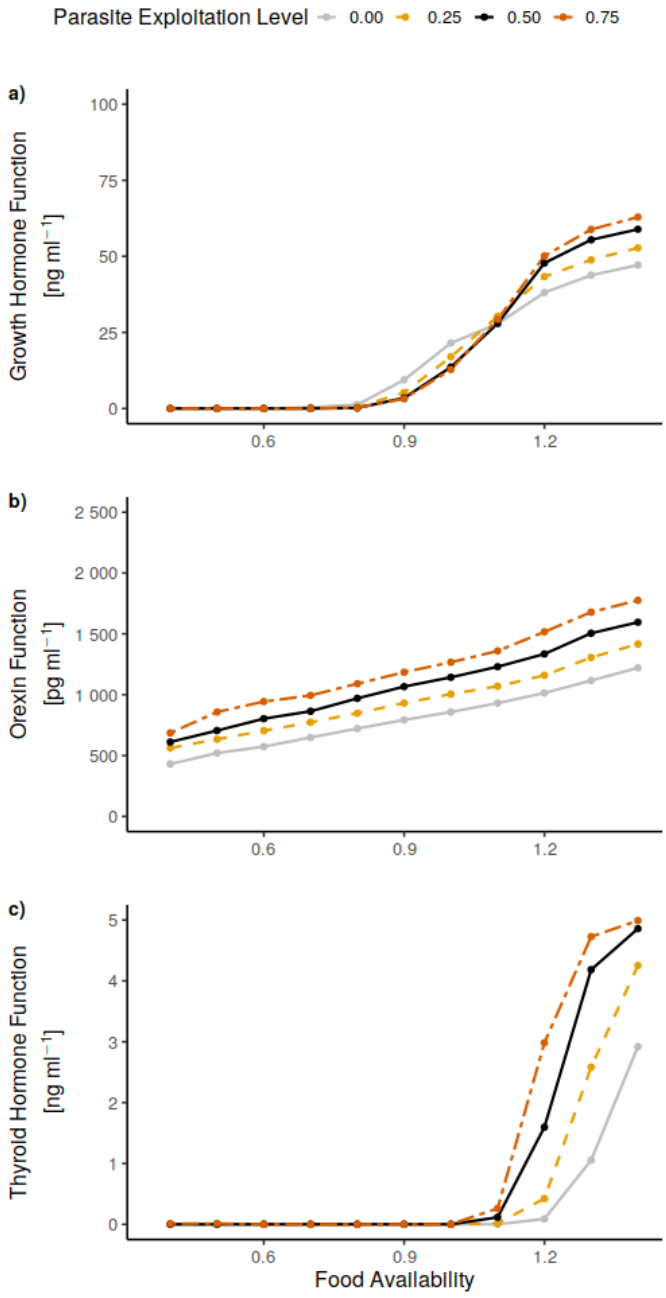

Figure 4: *Mean hormone function levels at different levels of food availability for model fish as a response to differing levels of parasite exploitation levels.*

used a purely phenotypic approach. Finally, for GHF we see something akin to a balancing point in **figure 4a**, as it was optimal for individuals with no parasites to have higher GHF levels at intermediate levels of food availability. When food availability was rich, on the other hand, it was optimal for fish experiencing the highest parasite exploitation level to have elevated GHF levels (**figure 4a**).

3.2 Using hormone strategies to prepare the phenotype for the future

In **Paper I,** where we looked at hormone strategies in constant environments, there was just simple preparation of the phenotype involved, because of the stable and predictable nature of the food availability. As a result of this, it was optimal for the model fish in **Paper I** to keep their reserves at a stable low level, using almost everything to pay for growth and other energetic costs. The same was also true for the individuals living in the constant environments presented in **Paper II**.

With the introduction of a variable environment in **Paper II**, it became optimal for individuals to have larger reserves. We also saw that it was optimal for fish to move mortality costs over time, by primarily foraging and growing in periods of high food availability, while reducing activity and saving energy during poor times. However, this strategy also lead to higher instantaneous mortality when the environment was rich. Fish that finished their growth periods faster (because they on average experienced a higher food availability) thus had both higher mean instantaneous mortality and overall survival, while the opposite was true for slow-growing individuals. In contrast, fish that lived in poor constant environments (from **Paper II**) experienced higher instantaneous mortality and lower survival.

In the variable environment in **Paper II** we also saw the optimal hormone strategy reflected in the use of reserves. It was optimal for fish to build their reserves when the food availability in the environment was at an intermediate level, as low GHF and THF levels paired with intermediate levels of OXF increased net energy intake (see **section 3.1**). When food availability was high, it was optimal for reserves to be used for growth and to pay for increased metabolism, due to the higher GHF and THF levels expressed here. We also saw that it was optimal for fish that had experienced poor food availability in the past to first use stored energy reserves, and only increase foraging when their reserves were close to depletion. When experiencing poor food availability, however, fish could no longer afford both an elevated metabolism and growth, and it therefore became optimal to use reserves to survive while waiting for the environment to improve. This was supported by low levels of OXF, and thus foraging, that kept reserves at a level that normally would avoid starvation. Only when the fish faced starvation after having experienced poor food

availability for an extended period, did it become optimal for them to increase their foraging activity by raising their THF and OXF levels, at the cost of a big increase in instantaneous foraging- and scope mortality risk.

When fish in addition to being in a variable environment also were exposed to parasites (**Paper III**), it became optimal for them to save more energy in their reserves than non-parasitised fish. This was because they have to account for not only their own energetic cost, but also for the parasites they harboured, to survive possible future periods of low food availability. However, we also found that highly parasitised fish had less in their reserves, than fish with an intermediate parasite load, for the majority of the growth period.

It should be noted that the patterns we see in how the hormone strategy prepares the phenotype for the future are the result of the autocorrelation level set in the model versions used. This is because the autocorrelation changes what can be expected in the future, and therefore the optimal hormone strategy for preparing the phenotype for what is to come (see also **section 4.3.3** p. 41).

3.3 A potential mechanism for parasite manipulation

While **Papers I** and **II** investigate optimal hormone strategies under constant or varying food environments, **Paper III** studies hormone strategies under the additional energetic costs of harbouring parasites. Specifically, we used the hormone model to investigate if simple mechanisms can lead to something that looks like advanced multidimensional parasite manipulation. We asked if increased energetic cost due to a parasite infection can lead to (1) changes in host hormone strategies, (2) changes in allocation to growth and reserves (i.e. host condition) and (3) an increased mortality due to predation, even in the absence of an explicit strategy by the parasite.

We found that it is optimal for fish with parasites to have increased hormone function levels (see **section 3.1**), resulting in increased activity, intake, growth and reserves (see **section 3.2**). A consequence of living according to the optimal policy for parasitised fish was also increased predation mortality due to higher required foraging activity. These are all signs that often are interpreted as effects of parasite manipulation. However, there is no direct route for parasite manipulation in our model; these results are all due to optimal host compensation strategies in response to increased energetic costs due to parasite infections.

Although we did not optimise the parasites' exploitation levels in the model, we could still calculate fitness proxies for the parasites: When assuming that we were dealing with a developing parasite (either because it is growing in an intermediate host or because it has

not yet finished reproducing in its final host), we found that an intermediate exploitation level was most beneficial to the parasite, as it best solved the challenge between extracting enough energy without killing the host (also referred to as the virulence-transmission trade-off, see for example Bull 1994 or Alizon et al. 2009). If we, however, assumed that we were dealing with a trophically-transmitted parasite that was ready to leave its host, we found that the parasite should exploit its host as much as possible to raise its transmission success, as this increased the probability that the host would get eaten by a predator. However, this should probably only be done up to a certain point, as extracting too much energy would lead to starvation in the host before the parasite can be transmitted.

3.3.1 Going beyond Paper III with parasites

In **Paper III** we only investigated situations where the host was optimised for the parasite exploitation level it was exposed to. This is not unrealistic, as the parasite-host relationships we see in nature often are the result of long co-evolutionary arms races between hosts and parasites. It has been shown in a range of species that the maximum observed level of virulence of a parasite usually is higher than the virulence that gives the parasite the highest fitness (Jensen et al., 2006; Alizon et al., 2009; Poulin, 2007). This can be investigated by letting the model host experience a parasite it is not adapted to, so that host behaviour is not optimised towards it. I did this by using the lookup table produced in the backwards iteration step of the simulations presented in **Paper III** (see **section 2.2** p. 21), which can be viewed as the adapted strategy of the host. While keeping everything else equal, I then changed the parasite exploitation level in the forwards part of the model, and hosts thus experienced lower or higher parasite exploitation levels than those they were adapted to. I did this in the range of parasite exploitation levels from 0.0 to 1.0 with increments of 0.005. However, it should be noted that an optimisation model is not the best tool to look at co-evolutionary games, so caution must be taken when interpreting the results in **figure 5**, as this analysis is at the very border of what the model can help us understand.

Unsurprisingly, hosts that are exposed to a parasite with a lower exploitation level than they are optimised for, experience a higher survival (**figure 5a**). Interestingly, within a limited range, fish that are optimised for lower parasite exploitation levels still have higher survival than the hosts optimised for higher exploitation levels, even when they are experiencing the same level of parasite exploitation. Thus, a cost of parasite defence (Schmid-Hempel and Ebert, 2003) emerges from the model itself, not in the form of a costly immune system, which is not included in the model, but in the form of a cost of compensatory behaviours. However, the gain in survival due to lower cost is only within this limited range, as hosts starve to death when exposed to higher exploitation levels

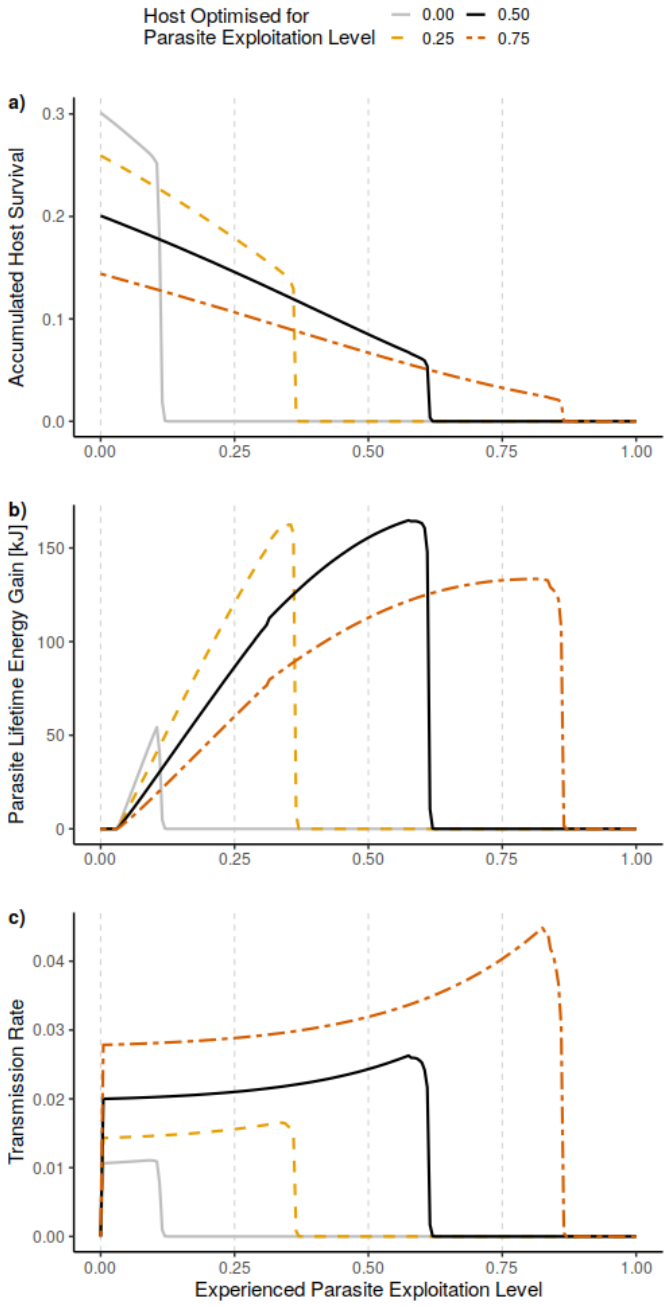

Figure 5: *Responses in hosts from the parasite's point of view when the fish host is exposed to differing parasite exploitation levels that they are not optimised for. Lines represent the means in the fish population. When accumulated survival (reflects the survival at length 30 in Figure 2a in* ***Paper III****) drops to 0 hosts die due to starvation, and subsequently the parasite lifetime energy gain and transmission rate also falls to 0.*

than they are optimised towards. Hosts with a lower defence are thus balancing on a tight rope between paying the defence cost and facing starvation. Hosts that can accurately identify the exploitation level and adjust their defence accordingly, should therefore have a selective advantage.

If we rather look at this from the parasite's point of view, **figure 5b & c** indicates that a mutant parasite that exploits the host more than the host is adapted to (here: optimised towards), will have higher transmission and parasite lifetime energy gain. This is because the host has reserves not only to pay the energetic costs for itself and the parasite when the food availability is rich, but also to survive potential future periods of low food availability. Since the environment is autocorrelated, the fish do not know how long a period like this would last, but is evolved to expect that it will improve in the future, so it builds up reserves accordingly. What is best for the parasite therefore seems to be to exploit energy from the host that represent normal environmental variation in addition to the parasite exploitation level that the host is adapted to.

In other words, it is not only the host that are balancing on a tight rope, but also the parasite: Taking too much energy could be fatal, leading to host starvation if a drop in food availability suddenly lasts longer than what could be considered normal. **Figure 5** thus seems to indicate that parasites should opt for a cautious strategy, and that the exploitation level that gives the highest parasite fitness probably is an intermediate one. However, as mentioned above, an optimisation model is not the best suited tool to look at the co-evolutionary game between parasites and host, so further investigation should be done using game theory (Maynard Smith and Price, 1973, but see Day and Proulx, 2004) or evolutionary individual-based modelling (Grimm and Railsback, 2005). Still, the virulence level of the parasite probably depends on the starting point of the co-evolutionary timeline (Nidelet et al., 2009; Cressler et al., 2016; Bull, 1994): If the hosts always die due to the infection, it would be unfavourable for the parasite, and it should evolve towards a lower virulence. It should probably, on the other hand, evolve towards a higher virulence if it could exploit the host more while still balancing the trade-offs between energy gain and host survival due to starvation or predation. But again, testing this needs to be done with a different modelling method than dynamic optimisation, as illustrated by the sudden drop in survival in **figure 5a** which is not very realistic in nature due to factors not included in our simplified model.

Overall, the resulting behaviour following a parasite infection, is one that potentially is adaptive for both the host and parasite: The host behaves optimally with regard to compensatory behaviour, and while not doing as well as parasite-free individuals, they still do much better than individuals not optimised to the same exploitation level. Parasites may be selected for different exploitation levels, leading to different levels of phenotypic

change in their host, depending on their life histories or developmental stage (**Paper III**). In general, both the parasite and host seems to do best when the host is optimised for the parasite's exploitation level (**figure 5**), but this needs further investigation. In this sense I fully agree with Lefèvre et al. (2008) that behavioural changes, even those that seem to primarily benefit either the parasite or the host, may have evolved as a compromise between host and parasite strategies, resulting in a truly mixed phenotype (Dawkins, 1982).

4 General discussion

> *"So I quoted the First Law of Mentat at her: 'A process cannot be understood by stopping it. Understanding must move with the flow of the process, must join it and flow with it.'"*
>
> Herbert (1965)

The work presented in this thesis represents an attempt to strengthen the link between proximate physiological mechanisms and ultimate evolutionary answers. We have done so by developing and using a dynamic optimisation model that optimises a simplified hormonal system in a generalised juvenile fish. By doing this we have ended up with a model that produces solutions that can be verbally explained using evolutionary theory. However, without the model we would not have had the insight to produce these verbal explanations, as we for example did not expect that fish would start growing faster if exposed to additional energetic costs by parasites (Figure 1 & 3 in **Paper III**).

During this work we have learned that hormones can evolve as a unified strategy that affects growth and survival in juvenile fish. This hormone strategy prepares the fish phenotype for the future in variable environments in addition to compensate for additional costs of parasitism. We have even found that parasites may affect the hormone strategies and behaviour of their hosts simply by the act of extracting energy. And we have done this by moving from the optimal endpoint of behaviour, to an optimal midpoint of physiology, where growth and survival are consequences of a unified hormone strategy.

To our knowledge the hormone model represents the first model approach to describe evolutionarily optimal hormone strategies towards a combined challenge of life history and environment. Our model is thus an addition to the already expanding toolbox of evolutionary endocrinology (Zera et al., 2007) and evolutionary biology in general. Even with its simplified hormone functions, the model still reveals patterns found in nature. It also has the potential to be used as a tool to find potential patterns that can later be investigated though experiments, like for example artificial selection, hormone manipulation, gene editing and observational studies. It can be used as a "computer laboratory" to help us answer questions, either in its current form or by being extended in new ways by adding new hormone functions, other aspects of the organism's physiology or environment or by modifying it to better fit the study of a specific organism.

4.1 Does it matter whether we optimise hormones and not behaviour?

Hormones provide a link between instantaneous behaviour and long-term fitness. They also provide a way to move our attention and explanations from optimal end points (the

phenotype), to optimal midpoints (mechanisms) from which growth and survival emerge. We also saw that hormones can potentially limit the number of behavioural options, as they have to work together as a whole for fish to successfully finish their growth periods. In our papers we also argue that the inclusion of hormones is a way to partly overcome the phenotypic gambit. But does it actually matter if we optimise the midpoint and not the endpoint?

In **Paper III** we wanted to look closer at what we refer to as advanced parasite manipulation of the host. The endocrine system is one part of this picture as hormonal changes have been observed in hosts that changes their behaviour following infection (see for example Escobedo et al. 2005). Hormones have also been proposed to be one of the mechanisms that parasites use to manipulate their hosts (Herbison, 2017). Without the hormones, the model would therefore produce less enlightening results.

In **Paper I** and **II**, however, the questions whether hormones matter or not are open for more discussion. On one hand the inclusion of hormones is important to show that hormone strategies can evolve in theory, on the other hand; are we just introducing a slightly different phenotypic gambit? Of our three hormone functions THF is the only one that affects more than one dimension of the physiology of our juvenile fish (see **section 2.1** p. 19). In principle GHF and OXF could be replaced with just growth and appetite directly, without affecting the results of the model. So does it matter if we optimise hormones or not behaviour directly? In order to investigate this, our hormone model needs to be compared with a new separate control model where we optimise behaviour directly. It should also be noted that the length of the time steps used might have an impact; if we were to choose a different time step length than a week, and/or let the hormone functions vary at different intervals, the emerging hormone strategies might be more conservative than those presented in this thesis. Still, the hormone model in its current form has been able to help us think about hormones in a new way; not as separate single hormones, but as part of a unified adaptive hormone strategy that helps organisms solve trade-offs and survive their growth periods. So even if three very simplified hormone functions are at the limit of how simple a hormone model can be, it is still able to affect our thinking in a way that a similar model without hormones would not be able to do. That the model can reproduce hormonal patterns that are reported in the literature is also promising.

4.2 How simplifications might limit research

In model and research in general we often work by breaking down bigger problems into smaller parts (see **section 1.1** p. 13). This simplification can be very helpful, and often

necessary for dealing with bigger complex questions. It is also very helpful to understand how each part works, before we try to put the pieces together. To be able to simplify is no doubt very useful and an important tool to gain understanding, but I think that sometimes we run into the problem of forgetting that these are simplifications and not necessarily how the world works.

Let us use my own thesis work as an example: When you read about phenotypic changes following infection, we are often faced with only three explanations: (1) The change is adaptive to the parasite, (2) the change is adaptive to the host or (3) the change is an side-effect of the infection and not adaptive for either the host or the parasite. These three single boxes can potentially make us forget about the alternatives, and can almost be rephrased into asking: Who is in charge of the host vehicle? Is it the host, the parasite or none of them? Most people working with parasites are of course aware that the situation is not this simple. In the case of rabies for example, it is well known that both the genes of the virus and the host have an effect on whether the behavioural outcome will be encephalitic (furious; the host becomes aggressive) or paralytic (dumb; the host gradually loses mobility motor control and consciousness), where the last type results in reduced parasite transmission (Lefèvre et al., 2009a). Words such as hijacking and co-piloting about the competition between manipulating parasites within a single host, further pushes our thoughts into a vehicle where only one or none can be in charge. If we, however, rephrase the question from a genetic point of view to: Which genes control the host vehicle? It becomes more obvious that what we could indeed be looking at a mixed phenotype (Dawkins, 1982) controlled by both the genes of the host and the parasite, which is closer to our results.

The problems caused by simplification are not unique for fields traditionally using more top-down approaches: Within the field of evolutionary endocrinology Niall (1982) made four rules for the evolution of peptide hormones, where number two was: "*Everything is made everywhere*", to bring to attention that every cell in principle can produce hormones as they have a full complement of genes. Later Luck (2014) added "*Don't be fooled by the labels you put on things*" to further emphasise this. Still we often simplify and assume that each hormone is produced just by a particular gland or tissue. It is also easy to forget that for example growth hormones affect other aspects of the organism than growth, like cognition (Prodam et al., 2012). This is less of a problem for researches within the field who are aware of this assumption, but for new students (or someone trying to read up on the field) these simplifications can potentially cause misunderstandings and problems.

We need to be aware of the more holistic view within research and be explicit when we make simplifications (be it top-down or bottom-up), even if we assume that everyone is aware of them. The phenotypic gambit (see **section 1.1** p. 13) is a prime example of

this as it has shaped the way we study evolution and ecology. I would therefore like to encourage my fellow researchers to be intentional and aware of the simplifications they make.

4.3 Future perspectives

In this section I will propose different ways of moving forward. I will specifically mention the hormone model, but further studies could also be done using different models or different methods entirely. Yet, whatever the methodology is, I think it is important to keep thinking about the combination of proximate mechanisms and ultimate explanations. Even going back to view old results with this frame of mind, might reveal something novel to us that we might have overlooked in the past.

4.3.1 Do we gain anything with increased complexity?

As discussed in **section 4.1**, to investigate whether it matters or not if we ignore hormones when studying behavioural responses, one needs to compare the hormone model to an almost identical model that optimises behaviour directly. I think moving forward a test like this needs to be done.

In addition to the hormone model the Theoretical Ecology Group also has an individual based model referred to as the AHA! (Adapted Heuristics and Architecture) model (Budaev et al., 2019). This modelling type (i.e. individual-based modelling; Huston et al., 1988; Grimm and Railsback, 2005; DeAngelis and Grimm, 2014) has often been criticised for being too complex and not precise enough as it is simulation-based (Kokko, 2007). The AHA! model now contains a simple hormone system (based on the one presented in this thesis), so it would be very interesting to see if a comparison between the hormone model and AHA! would yield the same results, and if we can learn anything new by the increased complexity that individual based models can offer. The AHA! model should for example be better at density dependence, but it also has a significantly longer running time than our dynamic optimisation hormone model.

4.3.2 How are hormone strategies affected by changes in parasite exploitation levels and predator mortality in variable environments?

In **Paper III** we only changed the exploitation level of the parasite and left other parameters mostly untouched (see **section 2.3** p. 24). I think it therefore would be interesting to at the same time adjust the predation mortality, and/or the importance of the strategy-dependent and strategy-independent mortality components like in Weidner et al.

(*manuscript*, see **List of publications** p. 9). I predict that adjusting both the parasite exploitation level and the mortality at the same time will yield differences in the time the model fish use to finish their growth periods, depending on which pressure is strongest.

4.3.3 How does the level of environmental variability affect hormone strategies and their associated mortalities?

From **Paper II** we saw that there is a difference between constant and variable environments. Specifically we saw that in stable environments the instantaneous mortality is higher when the food availability is low, while the opposite is true in variable environments. We also saw that individuals in variable environments saved more energy in their reserves. It would therefore be interesting to test this further by changing the level of autocorrelation in the environment and see how this affects the different mortality components and how the model fish use their reserves. A quick test I did using the hormone model indicated that it was optimal for fish in almost random environments ($E_{E\_autocorr} = 0.1$) to save slightly less in their reserves than in more autocorrelated environments ($E_{E\_autocorr} = 0.8$), but still more than in stable environments ($E_{E\_autocorr} \approx 1.0$). Still, this needs to be investigated more thoroughly with respect to hormone strategies, mortality components, the time the fish use to finish their growth periods, etc.

4.3.4 How do parasites associated with food availability affect foraging behaviour and hormone strategies?

Parasites can change the behaviour of hosts simply by being in the environment (Preston et al., 2014). Sheep are for example found to avoid more rewarding grass when this is associated with nematode infections (Hutchings et al., 2001). I think a natural step after **Paper III** (that looked at host compensation during infection), is to investigate these pre-infection defences in juvenile fish. A starting point could for example be to vary parasitism with food availability in the hormone model. However, in the sheep study they also found that among other things the immune status and parasite status of the animals significantly affected their foraging strategy (Hutchings et al., 2001). So it would also be a good idea to be specific about how many parasites infect the fish at each point in time, and their exploitation levels. One could then introduce a very simple immune system that assumes a certain number of time steps before one parasite is cleared by the system, or let hosts invest in this very simplified immune system to increase the clearing time. To do this it probably would make the most sense to include the parasite as a state in the model together with length, reserves and food availability. I expect that a strong

trade-off between foraging and infection would emerge, where for example we would see hosts primarily foraging where there are fewer parasites and avoid foraging in areas with high parasite risk unless they are desperate. However, if the cost of one parasite is not too high, I would also not be surprised if hosts with an optimal number of tolerated parasites emerge from the model.

4.3.5 New model extensions and hormones

As shown in **figure 1** (p. 18) the growth and thyroid hormones have other effects on the organism than growth, metabolism and oxygen use, the same is also true for the hormones affecting appetite. By including more effects of the hormones already included in the hormone model, the model fish would be faced with more trade-offs which potentially could affect the emerging hormone strategies. In this way the interplay between hormones might also appear more apparent, even though it will be harder to analyse than the model results presented in this thesis.

In addition to the hormone functions and their effects presented in this thesis, it would be interesting to also consider other hormones. Since we have only considered three of the four energy sinks of sexually reproducing fish (see **section 1.3** p. **16**), namely the basic maintenance of vital systems, somatic growth and parasites, I think it would be natural to start with including sexual development and expand with the immune system, as well as their associated hormones. This could potentially lead to finding interesting interactions between the hormones controlling these processes, as we know that for example sex hormones, like testosterone and oestradiol (Schmid-Hempel, 2011), are associated with the immune response in animals (Harris and Bird, 2000; Klein, 2006). In addition, the immune system is also proposed as a potential pathway for parasite manipulation (Escobedo et al., 2005). By including simplified hormone functions for both the immune system and reproduction this would open new possibilities to further our knowledge when it comes to sexual selection and parasite compensation in hosts, among other things. Still this should probably be done in a stepwise fashion by for example starting with the immune system and then move on to reproduction at a later point in time.

4.3.6 Moving away from optimisation modelling to study co-evolution

Like I mentioned in **section 3.3.1** (p. 33), to fully be able to study the co-evolutionary game between hosts and parasites we need to move away from optimisation modelling to game theory or individual-based modelling. I think a model that builds on the thinking of the proximate mechanisms and ultimate explanations would be very useful for this. One

such model is the individual based AHA! model (Budaev et al., 2019), briefly mentioned in **section 4.3.1**. I think it would be interesting to use this model, not only to investigate optimal virulence in the parasite and optimal compensation in the host, but also to study something as intimate as the evolution of personalities.

Animal personalities are usually defined as behaviour that varies among individuals, but that are consistent and predictable over time and across different situations within the individual (Barber and Dingemanse, 2010; Stamps and Groothuis, 2010). This variability has been found to be heritable (Bell et al., 2009) and many experimental studies have found a difference in the fitness of different personality types (see for example Dingemanse et al., 2004; Höjesjö et al., 2002). As with hormones, all four of Darwin's (1859) postulates are met and we should therefore expect that personalities also are under natural selection. In addition, it has been proposed that parasites might play a largely overlooked but important role in the evolution of personalities (Kortet et al., 2010; Poulin, 2013). One of the conditions for this is that there has to be a correlation between the personality trait in question and the cost of having a parasite infection. Such a relationship might exist as some studies have indicated that individuals with bold and exploratory personality traits may possibly have a stronger immune response and thus a lower cost of exploring than more shy and sedentary individuals (see for example Kortet et al. 2007). Kortet et al. (2010) and Poulin (2013) propose that there are two main ways that parasites might contribute to the evolution of animal personalities: (1) Directly selecting for personality traits that are related to the risk of getting infected, and (2) indirectly by affecting their host's fitness, as a consequence of their impact on the health and/or immunity of their host. I think investigating this in a co-evolutionary individual based model with both proximate mechanisms and ultimate explanations in mind would be incredibly interesting.

4.3.7 Including parasites in models

In this thesis I hope I have been able to show the importance of parasites in natural ecosystems, and how they can shape the life-histories and hormone strategies of their hosts. However, the majority of models still to my knowledge do not include parasites (excluding models where the parasite is a main focus), even if they include predators and prey in explicit detail. And if parasites are included they are often hidden within the background mortality, even though increased host mortality might not be the only outcome of a parasite infection. I would, therefore, like to encourage all ecological modellers to try to add even a simple parasite into their models. Even our super simplified parasite was able to change the optimal policy of the host, and thereby its survival and growth, and I am sure that there is much more to learn from existing models by the addition of a humble parasite.

4.3.8 Empirical research

As mentioned earlier in the methods of this thesis (**section 2.2** p. 21) modelling is a tool that lets us test our own thinking and logic, and we do not test nature in itself. The results in this thesis therefore need to be tested empirically either in the laboratory or in the field. When doing this, however, one should not expect to see exactly the same results as our model, as it is a very simplified representation of nature. Still the patterns indicated should hopefully be the same. And should the patterns not be the same, the empirical results can hopefully be implemented in a better future model. For as every researcher knows, research is not always about being right, but also about being wrong and learning from it.

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