Future challenges of salmonid aquaculture: sustainability and climate change

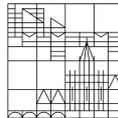**Doctoral thesis for obtaining the**

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Abbreviations

Aa	Amino acid		Kg	Kilogram
AP	Acidification potential		kWh	kilowatt hour
FES	Agilent Feature Extraction Software		kWp	Kilowatt peak
AB-PAS	Alcian blue-PAS		LCA	Life cycle assessment
NH_4^+	Ammoinum		L	Liter
NH_3	Ammonia		LW	Living weight
a. m.	*Ante meridium*		LC-PUFA	Long-chain unsaturated fatty acid
ANF	Antinutritional factor		CH_4	Methane
ADC	Apparent digestibility coefficient		µm	Micrometer
BLAST	Basic Local Alignment Search Tool		n-3-PUFA	n-3 polyunsaturated fatty acids
B.P.	Before present		NCBI	National Center for Biotechnology Information
BE	Belgium		NRC	National Research Council
Actb	Beta-actin		NIRS	Near-infrared spectroscopy
BSF(LM)	Black soldier fly (larvae meal)		NL	Netherlands
CO_2	Carbon dioxide		NO_x	Nitric oxide
CO_{2eq}	Carbon dioxide equivalent		N_2O	Nitrous oxide
CML(-IA)	Centrum voor Milieukunde		NO	Norway
FCKW	Chlorofluorocarbons		ODP	Ozone layer depletion
cDNA	Complementary Deoxyribonucleic acid		p	Parts
cRNA	Complementary ribonucleic acid		PE	Peru
DK	Denmark		PO_{4eq}	Phosphate equivalent
H_2O	Dihydrogen monoxide		PV	Photovoltaic
DLGAP4	Disks large-associated protein 4		PCR	Polymerase chain reaction
DM	Dry matter		*p. m.*	*Post meridiem*
EAA	Essential amino acids		PER	Protein efficiency ratio
Eef1a1	Eurokaryotic translation elongation factor 1, alpha 1		qPCR	Quantitative Polymerase chain reaction
			RAS	Recirculating aquaculture system
EU25/27	European Union with 25/27 member states		RoW	Rest of World (no Europe)
EP	Eutrophication potential		RT-qPCR	Reverse transcription-quantitative PCR
e.g.	*Exempli gratia*		Rps5	Ribosomal protein S%
FCR	Feed conversion ratio		SFA	Saturated fatty acids
FFS	Fischereiforschungsstelle		SI	Silicon

FM	Fish meal	SK-On Fil	Skin-on fillet yield
FO	Fish oil	SBM	Soy bean meal
FTS	Flow-through system	SPC	Soy protein concentrate
FAO	Food and Agriculture Organization of the United Nations	SGR	Specific growth rate
		m²a	Square meter per year
GLMM	Generalized linear mixed model	SO_{2eq}	Sulfur dioxide equivalent
GWP	Global warming potential	SO_x	Sulfur oxide
g	Gramm	TGR	Thermal-unit growth coefficient
GHG	Greenhouse gas	tkm	Ton-kilometer
GG	Guar gum	TFI	Total feed intake
HE	Haematoxylin eosin	CFC_{11eq}	Trichlorofluoromethane equivalent
HOG	Head-on gutted yield	USA	United States of America
HSI	Hepatosomatic index		
i.e.	*Id est*	TierSch-VersV	Verordnung zum Schutz von zu Versuchszwecken oder zu anderen wissenschaftlichen Zwecken verwendeten Tieren
ICP-MS	Inductively coupled plasma mass spectrometry		
IBW	Initial body weight	VSI	Viscerosomatic index
ISO	International Standards Organization	Y_2O_3	Yttrium oxide

Summary

Aquaculture is playing an increasingly important role in the sustainable production of protein-rich food. While companies producing the highly valued salmonids depend on cool, clear and oxygen-rich water, this might become an increasingly rare commodity due to climate change. Accordingly, this thesis focuses on the direct and relevant relationship between climate change and aquaculture. For this purpose, a high-resolution temperature dataset from five different fish farms is used to determine which external influences affect water temperature. Moreover, a niche model helps to ascertain the extent to which fish farmers in southern Germany will be affected by climate change in the future. Temperature dynamics are analysed under four projected climate change scenarios and show that solar radiation, air temperature and precipitation have a fish-farming-relevant impact on water temperature, depending on location. In the case of one fish farm, heavy rainfall events led to a rapid increase in water temperature, placing the welfare of the fish at risk. Calculations of different climate scenarios show that fish farms with high flow rates have a better chance of continuing production in the future. Based on the example of southern Germany, up to 77% of trout farms could be affected by the worst climate scenarios.

One of the potential measures suggested to combat water warming is to cover the raceways, which could provide additional benefits if the roofs are also used for photovoltaic systems, as investigated in the second study of this thesis. For this purpose, a life cycle analysis is used to determine the emissions of domestic trout production. An actual scenario is compared with two modelled scenarios with different levels of photovoltaic use and different proportions of fish meal in the feed. The findings suggest that trout production can be considered an environmentally friendly and resource efficient protein product with low CO_2 emissions of 1.2 kg per kg live weight. The majority of emissions originate from the use of oxygen and feed, while further research has shown that the use of photovoltaics in particular can save and compensate relevant emissions. For example, if 100% of the production area studied would be used for photovoltaics, CO_2 emissions could be reduced by almost 1 kg. The most interesting and surprising outcome is that a higher proportion of fishmeal reduces emissions. However, the results here are more complex in terms of ultimately judging the ecological input of fish meal due to difficult measurable side effects such as destruction of the seabed and overfishing.

As feed is the main source of emissions, an experiment was conducted to ascertain whether the protein content of feed – normally based on fishmeal or soymeal – could be replaced by insect meal. In this respect, five diets with different levels of insect meal replacing soy protein concentrate were used, while the nutrient composition was kept the same. The animals were not only monitored for growth performance, feed utilization and organosomatic indices but also physiological changes in the intestine and liver by histological and transcriptomic analysis. The results indicate better feed intake and improved growth in the insect protein fed groups. Conversely, the insect-free diet demonstrates a better protein retention and feed conversion ratio, indicating that insect meal is a good alternative for protein in fish diets.

The aim of this work was to improve knowledge of the interactions between aquaculture and climate change. The results showed that the main challenge is the warming of the rearing water. One solution is to shade the rearing system as well as the receiving waters. An additional benefit would be to combine this with photovoltaic systems to reduce emissions from the farm itself. Another factor found to have a significant impact on emissions was feed. Suitable and sustainable alternatives are currently being sought to replace the high-emission components of the feed. Insects are a promising candidate for this. The results of this work showed no negative effect of replacing soy protein concentrate with insect meal proteins on the health and growth of trout. If the insects are produced using renewable energy, they could therefore be a potential candidate to further reduce emissions and thus make aquaculture salmonids even more environmentally friendly.

Zusammenfassung

Aquakultur spielt weltweit eine immer größer werdende Rolle in der nachhaltigen Produktion von proteinreichen Lebensmitteln. Unternehmen, welche die insbesondere in der westlichen Welt geschätzten Salmoniden produzieren, sind abhängig von kühlem, klarem und sauerstoffreichem Wasser. Doch ebendiese Bedingungen werden durch den Klimawandel kontinuierlich seltener. Diese Thesis beschäftigt sich mit den unmittelbaren Zusammenhängen zwischen Klimawandel und Aquakultur. Hierfür wurde mit Hilfe eines hochaufgelösten Temperatur Datensets von fünf verschiedenen Fischzuchten einerseits ermittelt, welche äußeren Einflüsse sich auf die Wassertemperatur auswirken, ebenso wurde unter Zuhilfenahme eines statistischen Nischenmodells untersucht, wie stark Fischzüchter zukünftig in Süddeutschland vom Klimawandel beeinträchtigt werden. Die Temperaturdynamik wurde unter vier projizierten Klimawandelszenarien analysiert, wobei sich zeigte, dass Standortspezifisch vor allem Sonneneinstrahlung, Lufttemperatur und Niederschlag einen relevanten Effekt auf die Gewässertemperatur haben. Bei einer Fischzucht zeigte sich weiterhin, dass Starkregenereignisse zu einem rapiden Anstieg der Wassertemperatur führen, welcher das Tierwohl massiv gefährden kann. Die Modellierung der Klimaszenarien zeigte außerdem, dass besonders Anlagen mit einer hohen Durchflussmenge bessere Chancen haben, zukünftig weiter produzieren zu können. Die für Süddeutschland in der Fläche prognostizierten, schlimmsten Klimaszenarien wären eine Beeinträchtigung von bis zu 77 % der Forellen-Aquakulturbetriebe. Als potentielle Maßnahme gegen die Erwärmung des Wassers wird unter anderem eine Überdachung der Hälterungseinheit vorgeschlagen. Dies könnte noch weitere Vorteile bringen, speziell, wenn die Überdachung für Photovoltaikanlagen genutzt wird.

Mit exakt dieser Kombination hat sich die zweite Studie dieser Arbeit befasst. Hierbei wurden mit Hilfe einer Lebenszyklusanalyse (LCA) die Emissionen der heimischen Forellenproduktion ganzheitlich ermittelt. Verglichen wurden dabei ein Ist-Szenario mit zwei modellierten Szenarien mit unterschiedlichem Photovoltaik-Nutzungsgrad und verschiedenen Anteilen an Fischmehl im Futter. Allgemein zeigt sich hierbei, dass die Forellenproduktion mit einem CO_2 Ausstoß von 1.2 kg pro kg Lebendgewicht grundsätzlich ein umwelt- und ressourcenschonendes Proteinerzeugnis darstellt. Der Großteil der Emissionen wird dabei durch die Produktion von Sauerstoff und Futter erzeugt.

Weitere Untersuchungen zeigten, dass insbesondere die Nutzung von Photovoltaikanlagen zusätzlich Emissionen einsparen könnte. So könnte bei einer 100 %igen Nutzung der Produktionsfläche die CO_2-Bilanz um knapp 1 kg verringert werden. Unerwarteterweise führt auch ein höherer Fischmehlanteil zu niedrigeren Emissionen, wobei für die Ergebnisse hier schwer messbare Wirkungen, wie Meeresgrundzerstörung und Überfischung, zu berücksichtigen sind.

Da das Futter eine der Hauptquellen für Emissionen in der Aquakultur ist, wurde ein Versuch durchgeführt, um festzustellen, ob der Proteinanteil von Futtermitteln, welcher normalerweise auf Fisch- oder Sojamehl basiert, durch Insektenprotein ersetzt werden kann. Dazu wurde in fünf Futtermittel mit unterschiedlichen Anteilen an Sojaproteinkonzentrat dieses durch Insektenprotein substituiert. Die Zusammensetzung der Makronährstoffe blieb gleich. Neben dem Wachstum, der Futterverwertung und den organosomatischen Indizes wurden auch Veränderungen in Magen und Leber der Tiere mittels histologischer und transkriptomischer Analysen untersucht. Die Ergebnisse zeigten eine bessere Futteraufnahme und ein besseres Wachstum in den mit Insektenprotein gefütterten Gruppen. Umgekehrt zeigte sich bei dem insektenfreien Futter eine bessere Proteinretention und Futterverwertung. Dies deutet darauf hin, dass Insektenprotein eine gute Alternative für Proteine im Fischfutter ist.

Ziel dieser Arbeit war es, die Kenntnisse über die Wechselwirkungen zwischen Aquakultur und Klimawandel zu verbessern. Die Ergebnisse zeigten, dass die größte Herausforderung die Erwärmung des Aufzuchtwassers ist. Eine Lösung ist die Beschattung des Aufzuchtsystems und der Vorfluter. Ein weiterer Vorteil hierbei wäre die Kombination mit Photovoltaikanlagen, um die Emissionen der Fischzucht selbst zu verringern. Ein weiterer Faktor, der sich erheblich auf die Emissionen auswirkt, ist das Futter. Aktuell werden geeignete, nachhaltige Alternativen gesucht, um die emissionsreichen Komponenten des Futters zu ersetzen. Ein vielversprechender Kandidat sind hierfür Insekten. Die Ergebnisse dieser Arbeit zeigten keinen negativen Effekt durch den Austausch von Sojaproteinkonzentrat durch Insektenmehl-Proteinen auf die Gesundheit und das Wachstum von Forellen. Wenn die Insekten mit Hilfe erneuerbarer Energien produziert werden, könnten sie daher ein potenzieller Kandidat zur weiteren Reduzierung der Emissionen darstellen und somit Salmoniden aus Aquakultur noch umweltfreundlicher machen.

1. General introduction

1.1. Past and present history of global aquaculture

Aquaculture dates back thousands of years, with its earliest evidence found in China, where aquaculture began either with the combined use of ponds for water storage and carp during the Han Dynasty (2300-1700 BC), or even earlier, in 2500 BC, with Fan Li being reported as the first fish farmer (Beveridge and Little, 2002). Carp were later introduced to Italy by the Romans, while trout farming and breeding began in the 18th century (Bundeministerium Land- und Forstwirtschaft, Regionen und Wasserwirtschaft, 2024; Hofmeister, 2014; Tidwell, 2012). Since then, aquaculture has developed both technically and in terms of species diversity. Between 1870 and 1890, the rainbow trout was discovered as a valuable farmed fish and imported to Europe from North America (Stanković et al., 2015). In 1913, Joseph Hofer developed the first oxygen system for the live transport of trout (Patent D.R.P. 245509 1913). Technical developments especially in the feed sector with extrusion technique together with developments in domestication have enabled aquaculture production to significantly increase over the last 50 years. Since 1980, aquaculture production has increased from 4% of total fisheries and aquaculture production to 49% of total production, with 88 million tons in 2020, whereas fisheries production has slightly decreased since 2018 (FAO, 2022).

Asia is the leading region for aquaculture production, accounting for almost 92% of the world's total production (). The Asian market is dominated by cyprinids and omnivorous species, which account for a market share of 50% of world trade. On the other hand, salmonids have a relatively small market share but a market value of 18% of total production (FAO, 2022). The main producers of farmed salmon in the world are Norway – with a production of 1.47 million tons – and Chile (Aas et al., 2022a; FAO, 2022). Norway is also by far the largest producer of Atlantic salmon in Europe, followed by the UK and the Faroe Islands. Turkey dominates the market of rainbow trout, followed by Norway, Italy and Denmark (FEAP, 2021).

According to Brämick (2018), around 9,000 tons of rainbow trout and 6,000 tons of other salmonids are produced in Germany, which, despite the low numbers, has excellent physio-geographical conditions. Nevertheless, Germany is unable to meet its
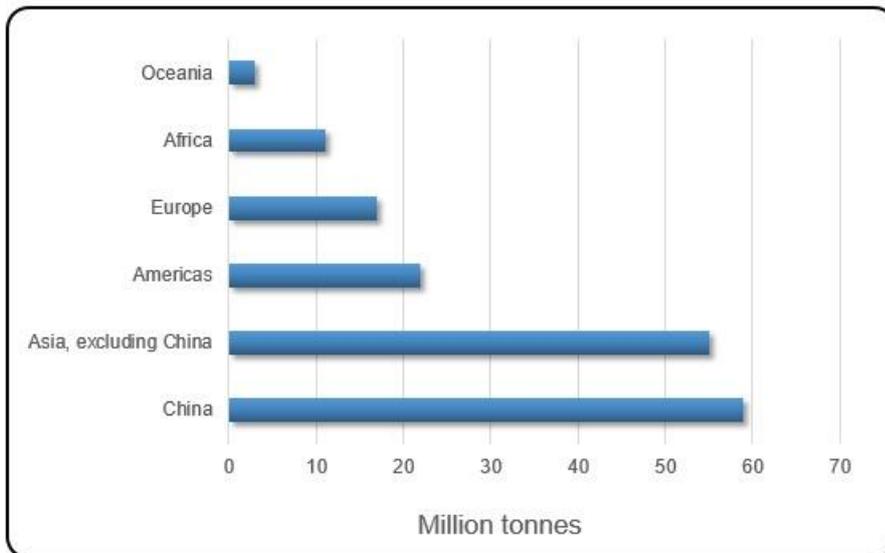
Figure 1 Regional contribution to global fisheries and aquaculture production according to FAO (FAO, 2022).

own demand for fish. With a self-sufficiency rate of about 13% for freshwater species and an import rate of 90% for seafood, there is evident room for improvement (Brämick, 2021). Although the government is making efforts to increase production and support aquaculture facilities, the existing regulations and high environmental standards are the main reasons why aquaculture production is not increasing (Brämick, 2021; Bundesministerium für Ernährung und Landwirtschaft, 2022). In fact, a slight downward trend has been observed in recent years (Brämick, 2021), which could intensify in the coming years when the consequences of climate change will force the sector to either make expensive renovations or reduce / stop production.

However, efforts to increase regional production would be beneficial, partly due to the fact that fish is a nutritionally valuable product, containing important macro- and micronutrients such as vitamins, iodine, selenium, and long-chain fatty acids (Tacon et al., 2020). Long-chain fatty acids were found to affect human health especially with regard to cardiovascular and mental health (Hibbeln et al., 2006). Omega-3 in particular is an important argument for the production and consumption of fish. Among other things, Omega-3 is important for children's development and has been shown to prevent coronary disease and the onset of Alzheimer's disease (Givens and Gibbs, 2008). One serving of salmonid fish provides on average 1.2-2 grams of Omega-3, significantly more than most foods and sufficient for human requirements (Givens and Gibbs, 2008; Kolanowski, 2021). However, as mentioned above, the production of these highly valued salmonids is threatened by the consequences of ongoing climate change (Collins et al., 2020; Ros et al., 2022).

1.2. Life cycle assessments

Agriculture – including aquaculture – is responsible for almost 20% of anthropogenic emissions, with 9.3 billion tons of so-called CO_2 equivalents in 2018 (FAO, 2020). Conversion to CO_2 equivalents is the main method used in life cycle assessments (LCAs), which balance the production processes, waste, energy and material flows required to manufacture a product (Figure 2). Based on these calculations, different products can be compared in terms of their environmental friendliness according to different impact categories (Finkbeiner et al., 2006). In addition, modeling can be used to identify particularly harmful production steps and subsequently improve them (Samuel-Fitwi et al., 2013a; Smetana et al., 2016). This can be achieved by using potential substitute processes and products, which offers considerable potential to make products more sustainable in the long run.

Different analysis methods assess different impact categories in an LCA, covering a wide range of climate affecting gases and future scenarios (Pelletier et al., 2007). The aim of these methods is to achieve the optimal calculation for a product by focusing on multiple outcomes, either in terms of short- or long-term effects, the destruction of land and / or water bodies or nature in general, or by focusing on the impacts on human populations (SimaPro, 2020).

The use and production of some resources in agriculture and industry can contribute to climate change, which in turn increases the energy and material inputs needed for agricultural / industrial production (Masson-Delmotte et al., 2021; Yohannes, 2016). This creates a vicious circle that can negatively affect aquaculture and increase the energy and technology needed. For example, when comparing CO_2 emissions from food production, meat and dairy account for the majority of emissions, while plant foods outperform most animal products, often with CO_2 levels below 1 kg CO_2/kg (Clune et al., 2017). However, finfish from global aquaculture emit approximately 4-6 kg CO_2/kg edible flesh, with rainbow trout produced in flow-through systems emitting 0.7-3.5 kg CO_2/kg live weight (d'Orbcastel et al., 2009; MacLeod et al., 2020; Samuel-Fitwi et al., 2013a, 2013b). Depending on different factors such as housing and feed, the production of beef emits approximately 26 kg, pork 5 kg and chicken 2 kg of CO_2 per kg live

Figure 2 Schematic representation of processes involved in a life cycle assessment.

weight (Arrieta and González, 2019; Clune et al., 2017; McAuliffe et al., 2016; Wiedemann and Watson, 2018; Wiedemann et al., 2017). Fish can therefore be considered a climate-friendly meat alternative. To date, it has been found that emissions in flow-through aquaculture are mainly from feed use but also from energy and oxygen use (Ayer and Tyedmers, 2009; Samuel-Fitwi et al., 2013a).

However, not all gases contribute equally to climate change. For example, methane – originating *e.g.* from livestock farming and nitrogen fertilizers – has a greater impact on the climate than CO_2, although it also decomposes at a faster rate. Accordingly, equivalents are created within a LCA to take such effects into account. For this purpose, the global warming potential of different gases is converted into a particular gas, often CO_2-equivalents (Acero et al., 2016). As shown in Table 1, methane (CH_4) has a factor of 28 because it contributes 28 times more to climate change than CO_2, and nitrous oxide (N_2O) has a factor of 273 (Helmholtz-Klima-Initiative, 2023). Consequently, emissions of 2 kg CO_2, 1 kg CH_4 and 3 kg of N_2O would be summarized as 849 kg CO_2-equivalents.

Another important factor in the LCAs of aquaculture are the fish reared. Carp for example can emit up to 6 kg CO_2 per kg live weight (Xing-Guo et al., 2023). Although they can be held extensively (with ~1.7 kg CO_2 per kg live weight (Clune et al., 2017)) in ponds, feeding mainly on detritus and insects, often they are fed additionally and dilute the water bodies (Dadebo et al., 2015; Xing-Guo et al., 2023). Tilapia, with 1 - 3 kg CO_2 per kg live weight, on the other hand can grow quite fast compared to salmonids, but require higher temperatures (Pongpat and Tongpool, 2013; Yacout et al., 2016). As carps, they are omnivorous fish and can therefore be fed with plant based

Table 1 100 year Global warming potential of selected greenhouse gases, hydrofluorocarbons and other chemical compounds, according to (IPCC, 2023a).

Name	Formula	GWP100
Carbon dioxide	CO_2	1
Methane	CH_4	27.9
Nitrous oxide	N_2O	273
CFC-11	CCl_3F	5560
Sulfur hexafluoride	SF_6	25200
Methyl chloride	CH_3Cl	5.54

feed, reducing their emissions (Tesfahun and Temesgen, 2018). A problem coming along with carps as well as tilapia is that the ponds often silt up. Methane gases can form in this sludge, increasing the emissions of the otherwise undemanding fish(Dong et al., 2023).But also the production system of salmonids can change the outcome of an LCA. Currently, two main production systems are used: open and closed systems (Tidwell, 2012). With particular regard to climate change and emissions from salmonid production, the main advantages and disadvantages of the different systems need to be clarified.

1.3. Environmental aspects of aquaculture systems

Open systems can be further subdivided into flow-through systems (FTS), ponds, cages and semi-recirculating systems. The advantage of FTS – which are commonly used in Germany – is the continuous supply of clear, cold and above all oxygen-rich water (Belle and Nash, 2008). In FTS, this supply is ensured by using water from rivers or streams, while cages float in lakes or the sea, resulting in optimal husbandry conditions for the fish with minimal energy consumption (Belle and Nash, 2008; Fornshell and Hinshaw, 2008). Although some might view the water flow through the system as water use in the sense of consumption, this must be viewed critically as the water is returned to nature without any real use (Fornshell and Hinshaw, 2008). Nevertheless, the water in a flow-through system is polluted by the release of fish feces and excess feed, which has the potential to eutrophicate the receiving water (Folke et al., 1994). For this reason, treatments such as constructed wetlands are often found at the outlet of intensive fish farms, removing most of the particles and nutrients from the water (Lin et al., 2002; Snow et al., 2012). In cages, especially where currents are not sufficiently strong to disperse fish waste over a large area, this can also lead to changes in the biomass and diversity of the benthos beneath and around the cages (Karakassis et al., 2000). As a result, cages are being moved offshore, where the waste is distributed more widely and therefore has less impact on the direct environment (Beveridge, 1994; Welch et al., 2019). In closed recirculating aquaculture systems (RASs), the treatment

5

and reuse of tank effluent reduces water consumption as well as the discharge of pollutants into the environment. However, the costs in both economic and environmental terms are high. The energy required to filter and treat the water and maintain its dissolved oxygen and water temperature at adequate levels is high and produces considerable emissions (Samuel-Fitwi et al., 2013a; Summerfelt and Vinci, 2008), such as CO_2, SO_2, P and N equivalents (Song et al., 2019). The (de-)nitrification processes during water treatment can also emit N_2O (Yogev et al., 2018).

A relatively new idea is the combination of the two systems presented here, namely the semi-recirculating system. In view of sustainability and ongoing climate change, it combines the environmental and economic advantages of both systems. Under optimal conditions, an adequate water supply ensures the operation of a flow-through system with little to no energy consumption. If water shortages are expected, the system can recycle the discharge water and pump it to the inlet of the rearing compartments to counteract a short-term water shortage. One prerequisite for cold-stenothermic aquaculture is a low inlet water temperature.

1.4 Feed – Not only emissions are important

A major contributor to emissions – apart from the energy requirements of the system used – is the feed used in salmonid farming (Aubin et al., 2009; Samuel-Fitwi et al., 2013a). As salmonids require a highly balanced diet specifically adapted to their physiological requirements, a balanced ratio of proteins (~35%) and lipids (~30%) as well as an appropriate processing of the ingredients used is required (Aas et al., 2022a; Dessen, 2018; Welker et al., 2018). While fish meal and fish oil require little or no processing, some vegetable ingredients such as soy require extensive processing to remove so-called anti-nutritive factors. At worst, these factors can cause enteritis and impaired growth (Dersjant-Li, 2002; Francis et al., 2001; Hendriks et al., 1990). Additionally, intensive crop cultivation uses many fertilizers and pesticides (Nemecek et al., 2011; Romero-Gámez et al., 2014). As some of the ingredients used have a high environmental impact, efforts are being made to find more environmentally friendly alternatives that can replace the high protein content without negatively affecting fish health and water quality (Hodar et al., 2020). A promising candidate are insects, as they are already a part of the natural diet of many fish species (Freccia et al., 2020). Furthermore, they can be reared at relatively low space, with a high reproductive rate, feeding

on wastes or by-products (Cadinu et al., 2020). However, the environmental friendliness of insect production comprises further relevant factors – *i.e.* feed, type of energy used, species, *etc.* – and it is not guaranteed that fish feed produced with insects is automatically more environmentally friendly than fish feed produced with fish meal or vegetables (Goyal et al., 2021; Smetana et al., 2019). Modahl and Brekke (2022) have found that proteins originating from insects fed with waste from vegetables can compete with other sources of fish feed proteins in terms of environmental friendliness. In this respect, black soldier fly larvae meal has been extensively investigated and appears to provide a better environmental balance and a comparable fish performance as commonly used protein sources such as fish meal or soy protein (Nairuti et al., 2021).

Diets for salmonids must not only be high in protein. The fatty acid composition must also be taken into account (Table 2) (FAO, 2024a, 2024b). In particular, n-3-polyunsaturated fatty acids (n-3-PUFAs) are essential for the growth performance and welfare of reared fish (Tocher et al., 2024). The majority of these in the diet are derived from marine sources (Sprague et al., 2017). Therefore, substitution with *e.g.* plant derived ingredients may affect the amino acid profile of the feed and therefore the fish themselves. Black soldier fly meal, on the other hand, already has a good amino acid profile, that can easily be further enriched with amino acids with the right diet (Čengić-Džomba et al., 2020).

The possibility of feeding insects algae or fish waste to enrich them with the much sought after Omega-3 fatty acid holds particular interest. If insects fed with Omega-3-rich diets were used to feed fish, the need for fishmeal and fish oil could be reduced, if not eliminated (El-Dakar et al., 2020; St-Hilaire et al., 2007a). This in turn could have a positive impact on the environmental aspect of salmonid production. It could also contribute to the optimal management of forage fish stocks. In the best-case scenario, the market could be relaxed to a level where stocks are not exploited to the maximum and / or illegal fishing is no longer profitable.

However, not only emissions play a vital role with respect to operating fish farms. Since the protein source of the feed is often a cost-determining factor, the protein source used within the feed determines the price of the aquaculture feed (Gómez et al., 2019). Many protein sources such as fish meal or plant alternatives such as soy are currently increasing in market value, since more likely and unforeseen extreme weathers can ruin or complicate the harvest (Fox et al., 2011; Markovic and Jovanovic, 2011; Mullon

et al., 2009). Therefore, future alternatives should be more cost-stable. With respect to price stability, lupines – for example – are considered a potential plant alternative since they are easy to harvest and have minimal environmental requirements (Szczepanski et al., 2022). Another promising candidate from this perspective are single-cell proteins (SCPs), which can be produced from a variety of easy-to-grow organisms, such as yeast or microalgae. Depending on the producing organism, different benefits are associated with these proteins. For example, SCPs produced by microalgae contain Omega-3 fatty acids, while SCPs produced by yeast are rich in vitamins and micronutrients (Jones et al., 2020).

Table 2 Amino acid (AA) requirements of Rainbow trout and Atlantic salmon compared to the amino acid profile of various feed ingredients (Fabregas and Herrero, 1985; FAO, 2024c, 2024d, 2024e, 2024f; Fuso et al., 2021; Mäkinen et al., 2016).

Species/Origin	AA requirement		AA Profile					
	Rainbow trout	Atlantic salmon	Herring meal	White fish meal	Soy	Wheat	BSF Prepupae	SCP Micro-algae
Unit	% min of dietary feed (Dry matter basis)		g/100 g Protein		g/100g		mg/g protein	g AA/ 16g N
Histidin	0.7	0.7	2.4	2	2.6	2.4	36.5	
Isoleucin	0.8	0.8	4.5	3.7	4.7	3	44	5.2
Lysine	1.4	1.4	7.7	6.9	6.4	2.7	57.5	9.3
Methionine	1.8	1.8	2.9	2.6	1.3	1.5	18.5	1.7
Phenylalanin	1.2	1.2	3.9	3.3	4.9	4.5	43	5.7
Threoine	0.8	0.8	4.3	3.9	3.8	3	39	4.4
Tryptophan	0.2	0.2	1.2	0.9	1.3	1.3	16.5	4.2
Valine	1.3	1.3	5.4	4.5	5	4.1	63	6.2

1.5. Research objects

To evaluate the effects of climate change on aquaculture and develop options to reduce emissions, establishing practical mitigation measures such as canopies to minimize temperature increases in the water or implementing partial circulation systems to counteract short- and long-term droughts are investigated. Furthermore, this study investigates the effect of abiotic variables such as air temperature, precipitation and shade on husbandry water temperature, aiming to fill important knowledge gaps on the effects of climate change on the cold-water aquaculture sector. The results will provide management options to help the sector to become more resilient to future challenges. A further focus is placed on the development of sustainable fish farming options and the establishment of eco-friendly fish.

In the light of the above, the main objectives of this thesis are to:

1. Assess and quantify the effect of different (environmental) variables on water temperature in aquaculture facilities.
2. Investigate the future development of water temperature with particular reference to four different climate scenarios.
3. Identify simple and cost-effective management solutions to cope with rising water temperatures in the future and reduce emissions on the farm.
4. Ascertain how aquaculture can be made more sustainable.
5. Assess the impact of insect-based proteins on fish performance and physiology.

2. Inland freshwater aquaculture in a warming world

2.1. Abstract

Climate change potentially threatens the sustainable production of highly valued cold water fish species in flow-through systems, such as salmonids. By analyzing the relationship of water temperature to hydrological characteristics, air temperature, solar exposure, and precipitation, this study predicted temperature dynamics of five temperate cold-water aquaculture facilities under four projected climate change scenarios. Air temperature was found to be directly associated with facility site water temperature, and based on rational assumptions, two of the five facilities were predicted to face critical warming by mid-century. Extreme precipitation events induced acute short-term increases in water temperature of up to 5 °C. Significantly lower warming, roughly equal to the projected climate change–induced increase, was seen with artificial shading lowering temperature by 1 °C. Complementary niche modelling revealed that 37-77% of current cold-water facilities will likely incur suboptimal climate conditions by the end of the century. Shading of raceways, more efficient water use, and disease management are proposed as key actions to pre-serve cold water aquaculture.

2.2. Introduction

Aquaculture has been identified as a key sustainable food producing sector as it has the potential to use renewable resources such as fish meal or soy for the production of protein-rich and healthy food (Cottrell et al., 2020; Gephart and Golden, 2022; Hilborn et al., 2018). The majority of these professional facilities are directly connected to, or strongly embedded in the aquatic environment. Therefore successful production largely depends on a stable water supply with limited variation in its physical conditions (Ebeling and Timmons, 2010). Especially in the production of stenotherm cold water fishes like salmonids, climate change might severely affect key water parameters. Consequently, in northern and central Europe, where the focus lies on this practice, aquaculture is particularly vulnerable to the impact of climate change (Brämick, 2021; Fornshell et al., 2012; Hough, 2022). Current climate change is expected to increase both frequency and intensity of drought and extreme rainfall events, and average temperatures in Europe are rising at a rate exceeding the global mean change of 1.09°C (Brasseur et al., 2017; IPCC, 2023; Wilke, 2013). Such extreme weather events affect water temperature and oxygen availability and influence, directly or indirectly, disease

susceptibility and growth of cultured animals (Fry, 1971; Pugovkin and G.R.I.N., 2020). Consequently, fish farming must adapt to such changing conditions as the future climate will exceed the historic climatic range in which aquaculture has developed in Europe (Gutiérrez et al., 2021; Osman et al., 2021).

Depending on intensity and temporal scale, changes in water temperature can exert both positive and negative effects on aquaculture. Temperatures in the range of 20 to 30°C are considered as incipient lethal temperatures, with temperatures above resulting in an acute thermal shock for most salmonid species. Still salmonids may acclimate to temperatures above their upper incipient lethal temperature when water temperatures increase slowly (Jonsson, 2023). Such long-term increases, which are considered to be of great importance in aquaculture, can be associated with climate change. Risks resulting from those increases are likely to outweigh any benefits of traditional cold-water fish farming. For example, while higher temperatures during winter are positively correlated with growth of rainbow trout (Morgan et al., 2001), warmer summers may be detrimental if water temperature exceeds the upper thermal limit for growth of the reared stock (Ebeling and Timmons, 2010; Jonsson, 2023). If high temperatures persist over an extended period, thermal stress can result in physiological changes that may negatively affect fish fitness (Alfonso et al., 2021). Warmer conditions, especially in summer, can cause changes in behavior and feeding efficiency and increase energy demand, leading to reduced growth and survival (Hjeltnes et al., 2008; Rottman et al., 1992; Wedemeyer, 1996). Excessive summer warming endangers cold water fish welfare and increases susceptibility to disease and pathogens (Alfonso et al., 2021; Schreckenbach, 2002). A striking example of this is proliferative kidney disease, an emerging disease in salmonids associated with high mortality at elevated temperatures (Ros et al., 2021).

Water temperature in freshwater aquaculture facilities is directly affected by air temperature and solar radiation on the water surface (Adam and Sullivan, 1989). Heavy rain on warm days may result in an acute rise in water temperature when water falling on heated surfaces runs into the water source, and fish stress can be exacerbated by increased inflow of suspended solids (Courtice et al., 2022; Jacquin et al., 2020; Zhao et al., 2018). Summer drought reducing the availability of water, also carries significant risks (Dabbadie et al., 2018). With low inflow of fresh water, concentration of metabolic end products like CO_2 and NH_3 might quickly exceed tolerable levels (Zhao et al., 2018). Additionally, water will remain longer in the system, absorbing more heat from

11

the surroundings. As a result, the water temperature will warm even faster (Adam and Sullivan, 1989).

The adverse effects of climate change on water supply and quality raise the question of the extent to which cold water aquaculture will be sustainable, and what measures might be undertaken to increase resilience of fish production to weather extremes, warming, and altered precipitation patterns. Few studies have investigated the consequences and risks of ongoing climate change on aquaculture. Given the rapid pace of such changes and the pivotal role of aquaculture in food production, this question needs to be addressed (Basen et al., 2022a, 2022b; Dabbadie et al., 2018).

Using a dataset with high temporal resolution, we assessed in this study the effects of current and projected climate change on intra-daily, daily and monthly temperature regimes in five typical cold-water aquaculture facilities in central Europe. In the first part, parameters that influence local temperature, such as differences in shading at the study sites, are described in relation to intra-daily and daily variations in weather conditions on water temperatures. A model was then created that plotted historical trends in monthly hydrological characteristics (air temperature, solar radiation and precipitation) against water temperatures at the sites. This model was used to test the impact of four climate change scenarios ranging from effective climate protection to worst-case global warming on cold-water aquaculture of the near future. To further test these relationships on a regional-spatial scale, the locations of professional fish farms in southern Germany were used to infer the environmental niche supporting that supports current land based cold water aquaculture. Niche parameters were projected to future climate conditions to assess the environmental suitability for land based cold-water aquaculture under expected climate change. Based on the warming trend in Europe, it was hypothesized that climate change has already caused water in aquaculture facilities to reach temperature thresholds close to the critical thermal maximum of salmonid species reared in aquaculture facilities (Perry et al., 2005). Findings and recommendations from this study will help inform decision making on effective adaptation and mitigation measures like increased use of shading in cold-water aquaculture in the face of rapid climate change.

2.3. Material and Methods

Study site characteristics. High resolution water temperature data were acquired from five flow-through salmonid aquaculture facilities (sites A to E) located in south-western Germany, an important central European salmonid farming region (Supplements, Figure S1). Study sites were selected to cover a broad range of environmental and production conditions. All facilities received water from a groundwater spring or headwater stream with multiple small springs before reaching facilities. Husbandry systems in fish holding facilities consisted of raceways with rearing compartments being either earthen or concrete ponds. Study site C has five natural ponds connected in line, while compartments of sites A, B, D and E consist of concrete basins. Study sites A and E each have a single raceway, B has two parallel raceways and study site D four parallel raceways. Raceways of sites D and E were completely shaded by a roof. The raceway volume ranged from 300 to 2000 m³ and flow rate through the system varied from 20 to 300 L/s (Supplements, Table TS1). These characteristics of the study sites, including calculations of energy input from the environment, and raceway volumes can be found in Table TS1.

Data collection and management. Water temperature (T_w, °C) at the sites was continuously recorded at one- or two-minute intervals. Data for all sites were available for the period from 2017 through 2021. For sites that recorded Tw in multiple compartments (B, C, D), only data from the most downstream compartment is presented, as these exhibit greatest warming. The data was used to

1) chart hourly and daily fluctuations in water temperature relative to local weather conditions;

2) investigate historic temperature trends by extrapolating the obtained relationships to historical weather data from the Germany's National Meteorological Service database (Deutscher Wetterdienst, DWD, https://opendata.dwd.de/, accessed July 2022); and

3) calculate future patterns of water temperature under projected climate change based on simulated weather data extracted from the WorldClim database (https://www.worldclim.org/data/index.html, accessed July 2022).

To investigate drivers of short-term fluctuations in water temperature, the following weather data were extracted from the DWD data portal (https://opendata.dwd.de; accessed July 2022): precipitation (P), solar impact (S), and ambient air temperature (Ta).

Data of local precipitation (mm/5 min) were extracted from georeferenced grids hourly. Heavy rain events were defined as precipitation ≥7.5 mm in 30 min, per the DWD definition (Deutscher Wetterdienst, 2023), with a cumulative sum of precipitation calculated backward and forward (R package data.table, function frollsum) (Dowle et al., 2015) at each data point. The difference between the two values was calculated.

Air temperature (°C) on a 5 min scale and sunshine duration (min/h) were obtained from the nearest weather station, and temperature data on a monthly scale were extracted from georeferenced grids available from the DWD data portal. Solar position (altitude above the horizon in radians) was calculated for each study site using the R package "suncalc" (Thieurmel and Elmarhraoui, 2019). Solar exposure in min/h was calculated by correcting sunshine duration for sun position using the formula sin(sun elevation in rad).

Extreme change in water temperature was defined as $\Delta t \geq 1$ °C within 30 minutes. In order to identify these events, a running average of the temperature was calculated over the data 30 minutes before and 30 minutes after each data point (R package data.table, function frollmean), and the difference between the two obtained values was calculated. Past and future estimates of water temperature were derived from the WorldClim historical monthly weather and future climate data (www.worldclim.org, accessed July 2022).

For future climate, four Shared Socioeconomic Pathways (SSP), SSP126, SSP245, SSP370, and SSP585, of the Max Planck Institute Earth System Model were extracted [MPI-ESM1.2 high resolution (Gutjahr et al., 2019)]. These scenarios cover a range from worst- to best-case climate change scenarios, with intermediate scenarios (SSP245 and SSP370) being the most realistic outcomes based on current climate conservation policies and pledges (Masson-Delmotte et al., 2021). In this dataset, precipitation (mm/month) as well as maximum and minimum air temperatures (°C) were available for each year from 1960 through 2017 and estimated for 20-year periods from 2021 through 2100.

Statistical analysis. Data was managed and analysed with R version 4.2 (R.Core Team, 2022) using packages "data.table", "forecast", "dplyr", "raster" (Hijmans, 2022), and "suncalc" (Thieurmel and Elmarhraoui, 2019).

To assess the effect of extreme precipitation events on water temperature, a pre- and post-event temporal analysis of extreme precipitation events and peak water temperature was carried out using local precipitation and water temperature averaged on a 5 min scale.

To assess the effect of artificial shading on water temperature, the diurnal temperature range (ΔT = wtmax - wtmin) in July and August was plotted with respect to shade (shaded vs. unshaded) and solar conditions (cloud vs. sun) in a linear model with interaction term and study site as random factors.

To test for drivers of daily fluctuations in water temperature, a linear auto-regressive integrated moving average (ARIMA) model was calculated by averaging data on a daily scale. The auto-regressive and moving average parameters correct for temporal processes of inhibition and attenuation in the temperature dynamics. Publicly accessible data were collected for local precipitation, air temperature, and solar exposure, calculated as sunshine duration corrected for sun position (duration x π/altitude). Sun hours and precipitation were lag-shifted with a factor 1 to obtain data of the previous day (lead function). The ARIMA was used to correct for autocorrelation in the high-resolution temporal water temperature models:

$$Y_t = \beta_0 + \left(\sum_{i=1}^{n} \beta_i X_{t_i} \right)^2 + \gamma_t$$

$$\gamma_t = \sum_{j=1}^{2} \varphi_i \gamma_{t-j} + \epsilon_t - \sum_{k=1}^{2} \theta_i \epsilon_{t-k}$$

where Y_t is a time series of water temperature, and $X_{t_1}, X_{t_2} \dots X_{t_n}$ are potential drivers of variation in Y_t. These include four dummy variables representing variation among the five study sites (each of levels 0 and 1) and three continuous variables: precipitation (P), solar exposure (S) and air temperature (Ta). Second order interaction terms were added by crossing each variable. β0 = coefficient of the intercept and β1 … βn are the corresponding model coefficients. The residual variation in water temperature Y_t was corrected for autocorrelation using ARIMA. The Bayesian information criterion

(BIC) was used to select the optimal ARIMA model. The final model included two orders of partial autocorrelation parameters, φ_i, and moving average parameters, θ_i. Discharge has been identified as an important variable in driving water temperature variation in free flowing water (Bal et al., 2014). However as flow was regulated at a constant rate in the different facilities (see Supplements, Table TS1) this effect is captured by the variation between study sites via the dummy variables in the model.

To estimate past and future water temperature based on available precipitation and air temperature data (historical weather data and projections from a global climate model), a linear model was calculated using the R standard function lm to predict monthly water temperatures:

$$wt = \beta_0 + \left(\sum_{i=1}^{4} \beta_i X_i \right)^2 + \epsilon$$

where wt = water temperature, X1 = location (factor), X2 = maximum air temperature, X3 = minimum air temperature, and X4 = precipitation. All second order interactions among predictors were included in the model.

Future environmental suitability for salmonid aquaculture. Complementary to the site-specific analysis, an environmental niche model (ENM) was used to describe the environmental niche supporting current salmonid aquaculture in temperate Europe and to assess environment suitability for salmonid aquaculture under projected end of century climate. Although ENMs are primarily used to forecast species distribution, they can be applied to socio-economic activity linked to distinct environmental conditions. Based on the extraordinarily long history of cold water aquaculture in southern Germany (Brämick, 2024), aquaculture facilities were considered to exhibit equilibrium distribution, *i.e.* they are commonly present in environmentally suitable areas and absent from those unsuitable, a fundamental assumption for correlative ENMs (Phillips et al., 2006).

The ENM was constructed in MaxEnt v. 3.4.1. and calibrated on locations of 107 cold water aquaculture facilities in southern Germany, compiled from publicly accessible information (Supplements, Figure S2). MaxEnt relies on a machine-learning maximum entropy framework to relate environmental conditions at farm sites to the background environment of the area obtained by sampling 10,000 random locations. The established relationships were used to characterize environmental suitability for cold water aquaculture throughout the region. In contrast to most other ENM methods, MaxEnt

does not require a priori assumptions about absences, allowing for seamless application to the presence-only dataset used in this study, and is more stable in the presence of correlated predictor variables (Elith et al., 2011; Merow et al., 2013; Wisz et al., 2008).

Environmental suitability for salmonid aquaculture was predicted from five bioclimatic variables and four landscape-related variables that were previously shown to affect distribution of temperate fish species (Basen et al., 2022a, 2022b) and obtained at a resolution of 30 arc seconds. Bioclimatic variables included isothermality (bio3), maximum temperature of warmest month (bio5), minimum temperature of coldest month (bio6), precipitation of wettest month (bio13), and precipitation seasonality (bio15). Data were obtained from the WordClim database (Fick and Hijmans, 2017) for near-current (1970–2000) and projected end-of-century (2081–2100) conditions across SSP126, SSP245, SSP370, and SSP585. Landscape-related variables included terrain roughness and northness (http://www.earthenv.org/ (Amatulli et al., 2018)); the human footprint, a composite measure of population density, built-up environments, and agriculture (Venter et al., 2023, 2016); as well as hydrological substrate, a categorical variable of four levels: pore aquifers, combined pore and fractured aquifers, fractured and karst aquifers, and aquitards and aquicludes (https://www.bgr.bund.de/had; HY1000 © BGR Hannover 2019). All of these variables were expected to influence potential environmental suitability for salmonid aquaculture and justification for inclusion is summarized in Supplement Table TS2.

All MaxEnt settings were left at default, with the exception of the regularization multiplier, which was set to 1.5 to provide better generalization of the model and to avoid overfitting (Basen et al., 2022a; Phillips et al., 2006). Model performance was evaluated by the area under the receiver operating characteristic curve (AUC). The AUC ranges from 0.5 (random prediction) to 1 (perfect prediction), with values 0.7–0.8 indicating fair performance and values >0.8 indicating good to excellent performance (Araújo et al., 2005). AUC was calculated for the final model, using all aquaculture facilities for training, and for 20 replicate runs fitted with cross-validation to assess performance on test data. The response curves of the predictor variables were inspected for plausible behavior, and the final model output for near-current conditions was visually checked for its power to capture the location of known salmonid aquaculture facilities. To calculate the proportion of current salmonid culture facilities that will remain in suitable areas under projected future climate conditions, the equate entropy of

thresholded and original distributions (EETOD) threshold was used to distinguish suitable from unsuitable areas.

2.4. Results

Intraday fluctuations in water temperature. In the two warmest months, July and August, daily variation in water temperatures (ΔT) at the study sites showed a steep increase from sunrise to 14.00 CET with distinct differences among study sites in night to day change on sunny days (Supplements, Figure S3). The increase in water temperature lagged about two hours behind the increase of air temperature. Study sites A and C showed the greatest ΔT, ranging from 2.43–3.73 °C, while sites D and E showed lowest, 1.60-1.78 °C (Table 3). On cloudy days, ΔT was lower at all sites compared to sunny days (Table 3).

Effect of shading on intraday temperature fluctuations. The impact of solar exposure on ΔT was modulated by artificial shading (Table 3) as indicated by a significant interaction term between shading and weather conditions (analysis of deviance with type II Wald F test: $F_{3,463} = 8.866$, $p = 0.03$). On sunny days, the intraday temperature increased less at shaded ($\Delta T[\text{shaded}] = 1.7 \pm 0.4$ SE) than at unshaded sites ($\Delta T[\text{unshaded}] = 2.7 \pm 0.3$ SE), whereas on cloudy days this difference in increase was less pronounced ($\Delta T[\text{shaded}] = 0.8 \pm 0.4$ SE and $\Delta T[\text{unshaded}] = 1.4 \pm 0.3$ SE).

Table 3 Number of observation days (Nsun/cloud), daily maximum water temperature (TMax; mean ± SD), and intraday increase in water temperature (ΔT; mean ± SD) relative to sun or cloud conditions at the five cold water aquaculture facilities (A–E) during July and August (2017–2021). Sunny days were defined as days with more than 11.8 hours of sunshine in summer and cloudy days as those with less than 1.7 hours of sunshine in summer.

Study site	N_{sun}	$TMax_{sun}$ (°C)	ΔT_{sun} (°C)	N_{cloud}	$TMax_{cloud}$ (°C)	ΔT_{cloud} (°C)
A	45	12.94 ± 0.67	2.43 ± 0.58	57	11.63 ± 0.46	0.92 ± 0.36
B	42	13.15 ± 0.81	2.00 ± 0.66	54	12.63 ± 0.93	1.17 ± 0.79
C	43	19.32 ± 1.43	3.73 ± 0.56	63	16.6 ± 2.10	2.07 ± 1.28
D[#]	49	10.56 ± 0.62	1.60 ± 0.53	80	9.66 ± 0.35	0.63 ± 0.26
E[#]	25	15.93 ± 0.96	1.78 ± 0.68	11	14.26 ± 1.41	1.01 ± 0.48

#: Completely shaded by a roof.

Effect of extreme precipitation events on water temperature. Extreme water temperature fluctuations, defined as >1 °C within 30 min, varied with study site (Table 4) and was highest at sites B and E, where it occurred on 2.7–6.0% of days in May through October, 2017–2021. Extreme water temperature fluctuations were rare (0.1 - 1.2% of days) at other sites.

The large majority (92.5%) of extreme precipitation events (>3.75 mm per 30 min) occurred May through October, on 2.8–3.9% of days. In most cases, extreme water temperature changes did not correlate with precipitation events, except for sites B and E, which showed 13.0% and 3.7 % overlap of these events, respectively.

Site B showed the greatest overlap of extreme precipitation with extreme temperature variation, and *a posteriori* analysis of these overlapping cases consistently showed a rain event (not always extreme) prior to each temperature increase. On average, 3 h 10 min were between peak precipitation and beginning of temperature increase (Figure 3a). Within an hour post-onset of these extreme temperature events, the temperature increased 1.5–5.0 °C, with the magnitude of increase being significantly positively related to the cumulative rainfall 3.5 to 2.5 h before the start of the temperature rise (Figure 3b; n = 26, R = 0.618, p < 0.001).

Table 4 Average frequency of extreme precipitation events and temperature increases at cold water aquaculture facilities (A–E) April through October, 2017–2021. Overlap indicates co-occurrence of events relative to the days with extreme precipitation events. NDays = number of days in analysis.

Study site	N_{Days}	Days with extreme precipitation events (%)	Days with extreme temperature increase (%)	Overlap (%)
A	917	3.37	0.11	0.00
B	920	2.83	5.98	12.99
C	888	3.48	1.23	0.00
D	918	3.91	0.22	0.00
E	592	3.91	2.70	3.70

Drivers of daily variation in water temperature. A daily time series dataset was compiled to analyze the impact of weather variables on water temperature at the five study sites. The dataset showed a significant residual auto-regressive component (Durban-Watson test DW = 0.47, p < 0.001), and ARIMA modelling revealed a 2nd order autoregressive process including two moving average terms. Correcting for these terms resulted in a significant explanatory fit between water temperature values and the predicted values from the ARIMA model (fitted vs. observed data: R²adj = 0.98, p < 0.001; ARIMA coefficients: AR1, AR2, MA1, and MA2: p < 0.001, Supplements, Table TS3).
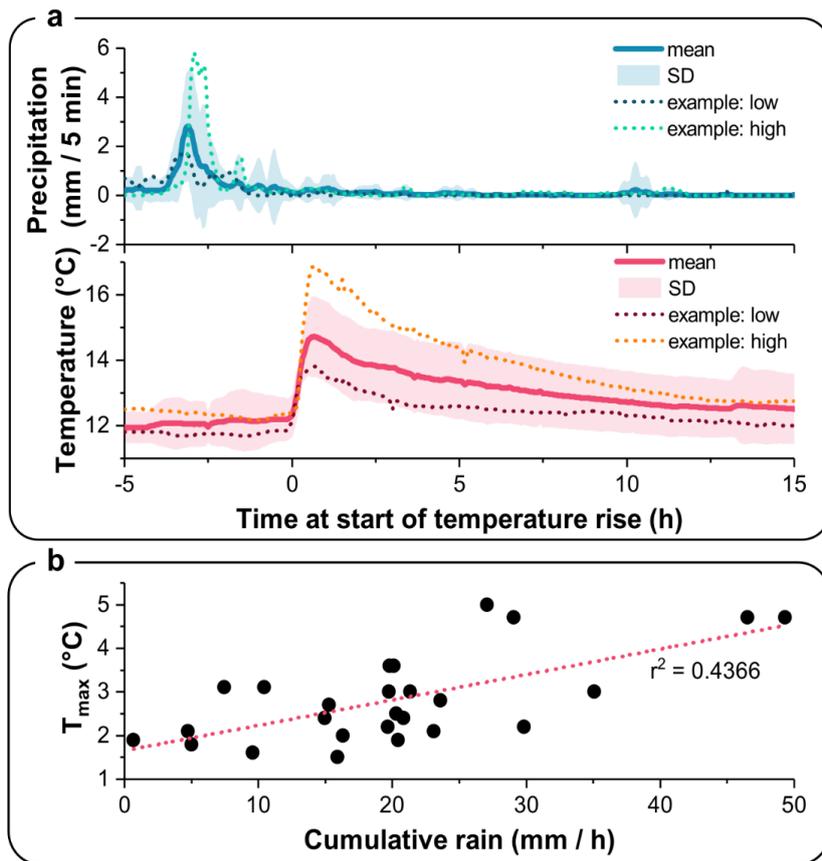
Figure 3 Relationship of precipitation with water temperature at study site B on days with water temperature increase >1 °C per 30 min.

a: Comparative time sequence of precipitation and water temperature (time set as zero at the onset of temperature increase; n = 26). Mean values of low and high extreme events (n = 3 and 4, respectively) of precipitation and temperature are shown as dotted lines. SD = standard deviation.

b: Correlation of peak water temperature (Tmax) with preceding cumulative rainfall (n = 26).

A significant and positive effect of air temperature, solar exposure, and a cooling effect of precipitation on water temperature was observed (summed z-values using Stouffer's method; air temperature, z = 49.5, p < 0.001; solar exposure, z = 5.60, p < 0.001; precipitation, z = -7.36, p < 0.001; Supplements, Table TS3).

Additionally, precipitation showed a significant interaction with air temperature (z = 3.04, p = 0.002; Supplements, Table TS3), which manifested as a positive effect of precipitation on water temperature at low air temperatures and a slightly negative effect of precipitation on water temperatures at high air temperatures.

The water/air temperature relationship differed significantly among facilities (Supplements, Table TS3). A post-hoc analysis with site-specific coefficients correlated with facility characteristics showed significantly stronger impact of air temperature at sites with lower discharge values (Spearman rank correlation, S = 40, p = 0.017) and lower flow rate through the facility (Spearman rank correlation, S = 39.5, p < 0.01). Solar exposure showed a near-significant negative relationship with artificial shading (Welch t-test, t = 3.13, df = 2.8, p = 0.058). No other relationships were significant (p > 0.1).

Past and future site-specific water temperature under climate change. The regression model explained about 91% of the variation in water temperature in the facilities over the course of the five-year study period (p < 0.001, n = 168, Supplements, Table TS4). Estimates of past water temperature based on local WorldClim climate data suggest that mean water temperature at the study sites has increased by 1 °C since the 1970s (Figure 4). Under projected climate change, site-specific water temperatures are likely to increase by up to 3 °C by the end of the century. The magnitude of predicted local temperature change generally parallels the magnitude of projected climate change, with the best-case scenario, SSP126, leading to a slight decrease in water temperature and the worst-case scenario, SSP585, resulting in the most extreme warming. Intermediate scenarios (SSP345 and SSP370) predict a temperature increase of <1 to >2 °C, depending on site.

Environmental suitability for salmonid aquaculture under climate change. The final MaxEnt model, using all aquaculture sites for training, featured an AUC of 0.74, while average test AUC for the 20 replicate runs with cross-validation was 0.65 (±0.11).
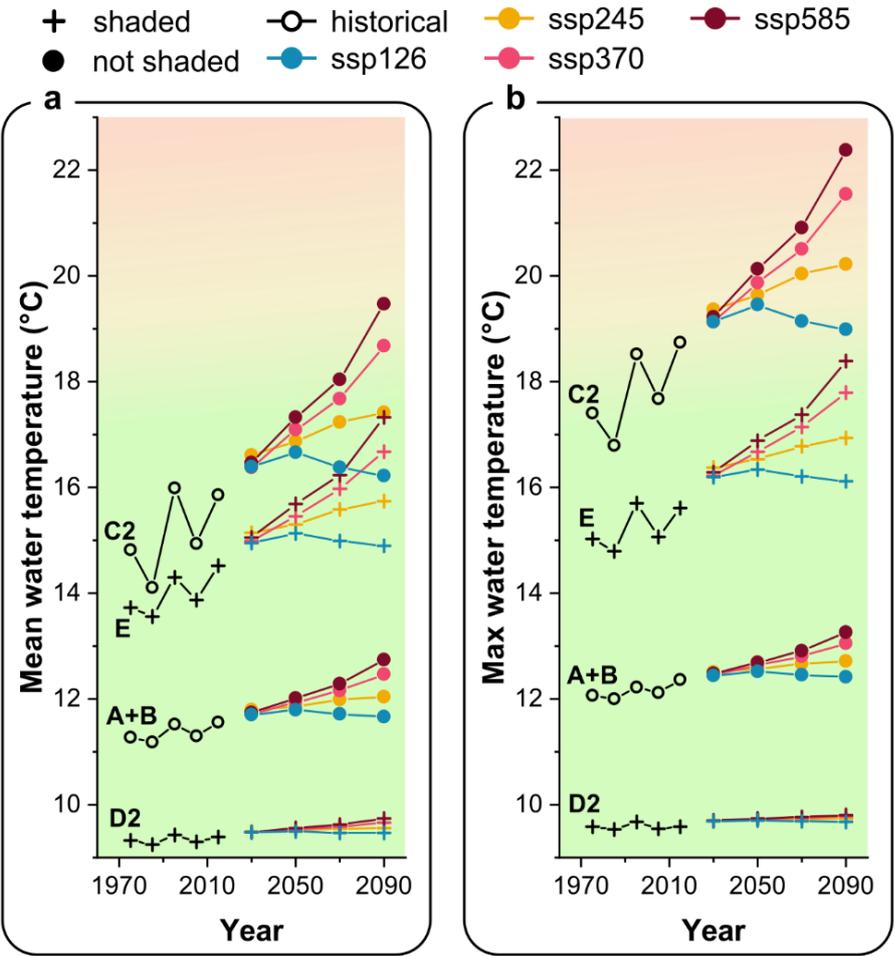
Figure 4 Estimated mean daily water temperatures July and August at the cold-water aquaculture facilities (A–E) under historic (1960–2017, data in 10-year increments) and projected future climate (2021–2100, data in 20-year increments) under climate change scenarios SSP126–SSP585.

a) mean daily temperature at the study sites;
b) mean maximum daily temperature at the study sites. Background fill indicates the temperature range for rearing juvenile and maintenance of adult rainbow trout (green: optimal temperature, red: detrimental/chronic stress temperature) (Jonsson 2023).

All response curves exhibit plausible, linear, or unimodal behavior (Supplements, Figure S4) and at the applied EETOD threshold, the final model was able to capture all of the known aquaculture sites, indicating overall poor to fair performance. The model predictions were primarily influenced by terrain roughness (permutation importance, 28%), bio13 (permutation importance, 20%), and human footprint (permutation importance, 17%). Temperature-related predictors (bio3, bio5, and bio6) accounted for 16% permutation importance.

Under projected climate change, environmental suitability for salmonid aquaculture in southern Germany is decreasing, with the magnitude of decrease being strongly affected by the applicable SSP (Figure 5). Under worst case and business as usual scenarios (SSP585 and SSP370, respectively), more than 70% of current aquaculture sites are likely to experience end-of-century climates that fall outside the niche that currently supports cold water aquaculture. This proportion decreases to less than 30% under the SSP245 scenario.
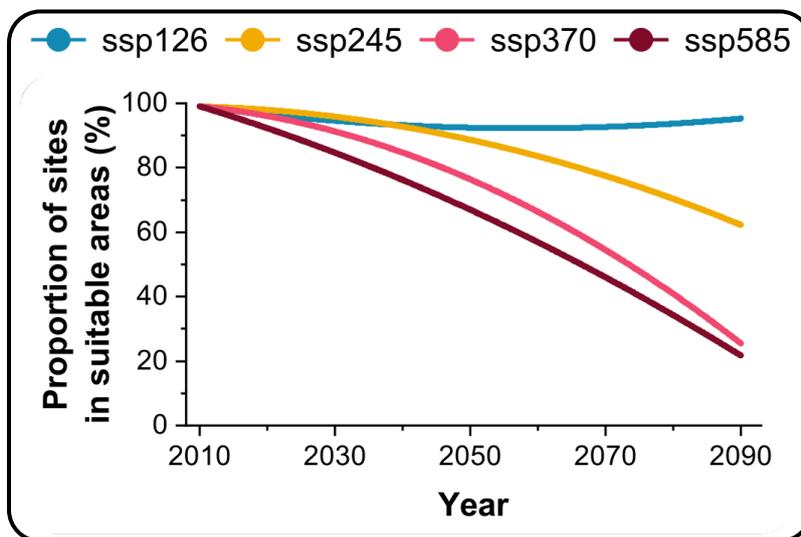
Figure 5 Proportion of present aquaculture sites (n = 107) in southern Germany within areas predicted to be suitable for cold water aquaculture (environmental niche model, AUC = 0.74) under projected climate change across four scenarios.

2.5. Discussion

This study quantitatively investigates the vulnerability of land based cold-water aquaculture to ongoing climate change and addresses its potential mitigation. Significant climate-related risks to traditional production of cold-water salmonids were identified. As expected, site-specific water temperatures have increased since the previous century and can realistically be assumed to approach critical thresholds in the more exposed facilities by the end of this century. Further, extreme precipitation events, predicted to increase in frequency and intensity, are shown to induce acute short-term increases in water temperature up to 5 °C, making rearing conditions unstable. Consistent with the site-specific analyses, generic niche modelling suggests that a consid-

erable proportion of cold-water facilities are likely to face end-of-century climate conditions incompatible to salmonid culture. Far-sighted effective adaption is a key challenge for continuing salmonid production.

Temperature-related risks. Rearing of salmonids, rainbow trout in particular, is optimal at 10–19 °C (Ebeling and Timmons, 2010; Jonsson et al., 2001; Myrick and Cech, 2004). Without mitigation measures, climate change is likely to place exposed facilities outside this range.

While rearing of juvenile trout is most efficient at 10–15 °C, optimal growth of adult trout without negative health effects is attained at 13–17 °C (Ebeling and Timmons, 2010; Jonsson, 2023; Myrick and Cech, 2004). Temperatures above 17 °C are manageable for most salmonids when oxygen supply is sufficient, while temperatures above 20 °C are unsuitable for most salmonid species (Ebeling and Timmons, 2010; Myrick and Cech, 2004), although there is restricted possibility for temperature acclimation. Experiments showed that fish reared in warmer environments are more resistant to the physiological effects of higher water temperatures (Jonsson, 2023). Since juvenile fish require cooler conditions, they are usually reared near the raceway inflow, with adult fish kept near the outflow where water is warmer, and the load of metabolic end products is higher.

At the five study sites, daily mean water temperature typically stayed within the 9-17 °C range (Supplements, Table TS5). During the warmest months, July and August, temperature increase on sunny days could reach 3.7 °C, resulting in areas with maximum temperature of 19 °C. Warming of >2 °C in an hour was occasionally recorded at all facilities. These peak temperatures and rapid fluctuations constitute a significant management challenge, as metabolic rate in fish is closely tied to water temperature, and warmer water increases fish oxygen requirements while reducing oxygen solubility (Johnston and Dunn, 1987; Xing et al., 2014).

The ARIMA modelling showed air temperature, solar exposure, and precipitation to be the chief drivers of daily water temperature fluctuation, and the regression analysis corroborated air temperature and precipitation as major drivers of monthly mean water temperature. Water temperature was in all cases positively correlated with air temperature, although the precise relationship of weather conditions with water temperature was site-specific and dependent on facility parameters like flow rate and shading. Since detailed data on discharge and water volume were missing, those factors were not considered in the ARIMA model. Nevertheless, their effect can be tremendous.

Discharge can vary with precipitation and result in a different pace of water warming, especially during sunny days. The more water flows through the system, the less it will warm within a certain time. Same holds for the water volume. The bigger the water volume, the longer it takes to heat the water.

Warmer air is a predictable consequence of climate change. The studied aquaculture sites were estimated to have already undergone an increase in water temperature of ~1 °C since the 1970s, coinciding with an average air temperature increase of the same magnitude. During the study period 2017–2022, the 30-year moving average of air temperature in south-western Germany has increased by a further 0.2 °C. Given the current rate of global warming and climate policies and pledges, the intermediate scenarios SSP245 and SSP370 are probably the best indicators of climate in upcoming decades (Hausfather and Peters, 2020; Raftery et al., 2017; Sherwood et al., 2020). Under these scenarios, the extent to which study site water temperatures are predicted to further increase varies. Sites A, B, and D are projected to experience negligible to moderate warming. As long as groundwater remains available [not guaranteed (Riedel and Weber, 2020)], water temperature will remain within the range for rearing salmonids, with possible beneficial warming (Elliott, 1976). At sites C and E, by contrast, water temperatures are likely to rise by 2 °C in summer, exceeding the optimal range for cold water aquaculture. The environmental niche model indicates that 37–77% of current cold water aquaculture facilities in southern Germany are likely to be located in areas of suboptimal climate conditions by the end of the century. This does not necessarily imply that cold water aquaculture will be impossible at these sites, but clearly indicates incompatibility with business-as-usual.

Precipitation-related risks. Strong rain events resulted in changes in water temperatures in two study sites with the strongest effect at study site B. At this site, short-term increases in water temperature (>1 °C within 30 min in summer) were consistently preceded by strong precipitation occurring roughly three hours prior. In one case, an increase of water temperature of 5 °C in an hour was recorded. An explanation could be the broad extent of impervious surfaces at the drainage area of B in contrast to the other sites. In a heavy rainfall event, this can lead to high accumulations of warmed run-off water. Warmed water entering the headwater stream that feeds the facility at site B abruptly increases water temperature in the rearing system. Increased frequency and intensity of strong rain events also carries the risk of flooding of the farm with fatal consequences for the fish (Wang et al., 2022).

At the other extreme, drought (*i.e.* low precipitation and/or increased evaporation) is also likely to negatively impact rearing conditions at the study sites, as evidenced by the ARIMA analysis. Low precipitation will result in low discharge and consequently lower flow rate at the facilities. Under this condition air temperature will have a greater effect on water temperature. Drought conditions, with reduced water supply, induces additional heat stress close to the critical thermal maximum for the salmonid species in the rearing system, especially in summer when air temperature may already be critically high. Flow rates lower than normal could also increase the concentration of suspended solids and toxic metabolic end products produced by the fish stock, since the water exchange rate is reduced, adding another layer of stress to the system (Becke et al., 2019).

A factor difficult to capture in the present risk assessment is how projected changes in precipitation affect long-term water availability for aquaculture facilities. Seasonal shifts in precipitation can diminish streamflow in summer, reducing water availability for facilities that are fed by surface waters. Sites fed by groundwater are likely to be more resilient, although availability of groundwater may be affected (Changnon et al., 1988). Groundwater levels show wide local variation, but that of most aquifers in Germany have declined during recent drought years (https://github.com/correctiv/grundwasser-data, accessed February 2023).

Synthesis and impact of climate change risks. Climate change has ramifications at short-term weather extremes creating temporary disruptions in water temperature. Additionally, long-term warming may increase vulnerability to summer temperature peaks and may eventually push conditions in currently operating facilities beyond the upper thermal limits for salmonid production. The short and long term scales are interrelated, as a warming atmosphere leads to a steady increase in the frequency and intensity of weather extremes, droughts, heat waves, and heavy rainfall events (Masson-Delmotte et al., 2021).

Extreme short-term warming is of particular concern for salmonid welfare, since acute thermal stress and resulting increase in metabolism will elevate respiration rate (Johnston and Dunn, 1987). According to Wedemeyer, 1996, respiration rate of stressed (active) salmonids can be eight times that of resting fish. Aeration systems may be unable to cope with such a rapid increase of oxygen demand, especially since higher temperatures reduce capacity for holding dissolved oxygen (Rounds et al., 2013). Consequently, oxygen levels could quickly drop below the recommended minimum for

salmonids of 6 mg/L (Ellis et al., 2002). Additionally, the increased metabolism may boost excretion of substances like NH_3 and CO_2 to toxic levels (Nakada et al., 2007; Wedemeyer, 1997).

A temperature peak coinciding with low water levels, as is expected during drought in summer, worsens the situation. With a constant biomass in the rearing system, concentrations may rapidly approach critical levels (Zhao et al., 2018). For example, with a water inflow reduction of 50%, NH_3 and CO_2 concentration are calculated to double, resulting in an increase of CO_2 from 20 mg/L to 40 mg/L (Supplements, Formula/Table TS6). Concentrations of CO_2 >30 mg/L can inhibit fish capacity to excrete CO_2 into the water, leading to accumulation in the blood to toxic levels, potentially resulting in lower growth and increased mortality (Ebeling and Timmons, 2010; Good et al., 2010; Hafs et al., 2012; Randall and Tsui, 2002; Schreckenbach, 2002). Even at ammonia concentrations below 0.05 mg/L, the level considered hazardous for salmonids, reduction in fresh water intake can lead to excess concentration that may cause adverse health effects by inhibiting the ability to excrete ammonia (Ebeling and Timmons, 2010; Randall and Tsui, 2002).

Another concern are climate change driven diseases. With low water quality and temperatures exceeding the optimal range, pathogen load may increase and disease resistance may be compromised (Alfonso et al., 2021). A prime example is proliferative kidney disease (PKD) in young salmonids. The causative agent of PKD, the myxozoan parasite *Tetracapsuloides bryosalmonae*, exhibits a complex lifecycle reliant on bryozoans and salmonids (Ros et al., 2022). The parasite is widespread in northern Europe and North America where it grows and produces spores in waters above 10 °C (Tops et al., 2009). Its primary host, bryozoans, can introduce the parasite into aquaculture systems. Infection of salmonids does not result in disease and mortality at water temperatures under 15 °C (Bettge et al., 2009). The parasite must be kept out of systems in warmer facilities to prevent significant mortality (Clifton-Hadley et al., 1986; Feist, 1993).

Climate change adaption and mitigation. Climate change is likely to result in water temperature dynamics that are outside the range of historic salmonid culture. Managers must prepare for projected conditions by considering currently available methods and innovations to establish resilience in the face of warming water and extreme weather events.

This study found evidence that shading of a facility contributes to cooling roughly equal to the projected temperature increase. This is in line with ecological studies reporting cooler water in areas shaded by riparian vegetation and forests (Bachiller-Jareno et al., 2019; Beschta, 1997; Garner et al., 2017; Rutherford et al., 1997; Spittlehouse et al., 2004). Kalny and colleagues (2017) observed a difference of 4.2 °C in daily maximum temperature of shaded and unshaded areas of the River Pinka in Austria. Depending on intensity and temporal scale, changes in water temperature can exert both positive and negative effects on aquaculture. The upper limit of lethality is considered to be 20 to 30 °C, which causes acute thermal shock for most salmonid species. However, if water temperature is slowly increased, salmonids can adapt to temperatures above their upper incipient lethal temperature (Jonsson, 2023). While study site D, which is completely roofed, does not heat up even on hot sunny days, the unroofed study site C shows intense warming during the day. Artificial shading provides protection from avian predators (Curtis et al., 1996), and the surface can be used for photovoltaic systems, reported to reduce the environmental impact of an aquaculture facility by more than 50% (Brinker and FFS Langenargen, 2019; Wind et al., 2022). The renewable energy generated can be used to offset aeration costs, and facilities could set up partial recirculation systems by treating and returning wastewater to the rearing system to compensate for reduced water supply (Sindilariu, 2007). This approach will help to prevent excessive concentrations of pollutants when available water is reduced, or fish metabolism increases due to elevated water temperatures. Combined with optimized feed quality and solids removal, pollutants in the water can be significantly reduced (Schumann and Brinker, 2020; Sindilariu, 2007). As Summerfelt and colleagues have shown, temporary recirculation is conducive to higher production than can be obtained by a serial reuse system (Summerfelt et al., 2009; Vinci et al., 2004). With respect to fluctuations in water availability, design of a filter system should consider the limited space in most fish culture facilities and additional operating costs of the filter system.

The adaption measures outlined above also address the risk of climate change mediated disease by keeping rearing conditions within optimal parameters. With respect to PKD, a system has been developed in the UK in which juvenile trout are briefly exposed to the parasite and subsequently kept in water <15 °C (De Kinkelin and Loriot, 2001). At such temperatures, the fish will acquire resistance to the parasite, protecting them in warmer rearing water (Ros et al., 2022).

2.6. Conclusion

The climate crisis holds significant challenges for temperate land based cold-water aquaculture. Foresight is crucial to continue the long history of professional salmonid farming within the 21st century, especially regarding the limited room for temperature adaptation in the commercial species. Shaded raceways, more efficient water use, and disease management are key actions to increase climate resilience. The boldest solution to climate and geographic independence of the sector would be to replace traditional land-based freshwater systems with recirculating aquaculture systems. However, while it is a viable option under certain scenarios, on a large scale such a solution would come at significant economic, energy, and ecological costs, rendering it incompatible with social, sustainability, and climate mitigation goals. Therefore, it is only with successful climate change adaption that the sector may develop as envisioned in the CERES report, which forecasts salmonid aquaculture to increase and to constitute important and environmental friendly food production in the future (Peck et al., 2020).

2.7 Supplements
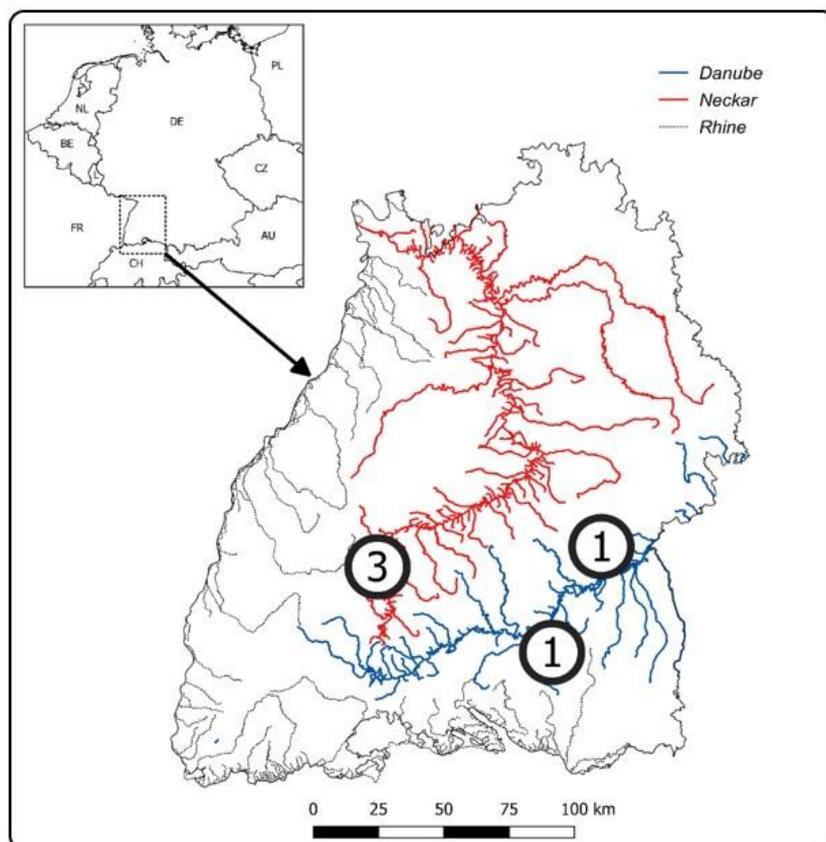
Figure S1 Circles give the approximate location of the five study sites along the river systems Danube and Neckar, Germany. Values in the circles respond to the number of facilities in that area.

Table TS1 Study site characterization. $\Delta T_{MAX/MIN}$ = Maximum / minimum temperature difference. Italic = shaded study sites, EI = Energy input from environment.

Study site	Flow rate [m³/s]	Distance to water source [m]	Water source	Raceway volume [m³]	ΔT_{MAX}[a]	ΔT_{MIN}[a]	EIsun (kW)	EIcloud (kW)
A	0.06	800 m	Spring	300	2.35	0.95	1220.64	462.13
B	0.18	500 m	Spring	1440	1.04	0.54	3103.92	1763.14
C	0.02	400 m	Spring	1189	3.36	1.11	624.55	346.60
D	0.30	0m + 300 m	Spring	1340	1.41	0.51	4018.56	1582.30
E	0.06	Max 2000 m	River with small springs along distance	2000	1.34	0.76	673.11	507.34

[a] The maximum and minimum daily fluctuations were calculated for July and August during sunny days and are mean values for the observed time period on sunny and cloudy days.

29

Formula for TS1:

A simplified model was used to calculate the daily energy input (*EI*) of environmental factors with the formula

$$Energy\ input = \frac{c * m * \Delta t}{time}$$

With c = specific heat capacity of water (kJ/m³ x K); m = water flow rate in m³/day (24 h); Δt = temperature change; time = solar exposure (12 h).
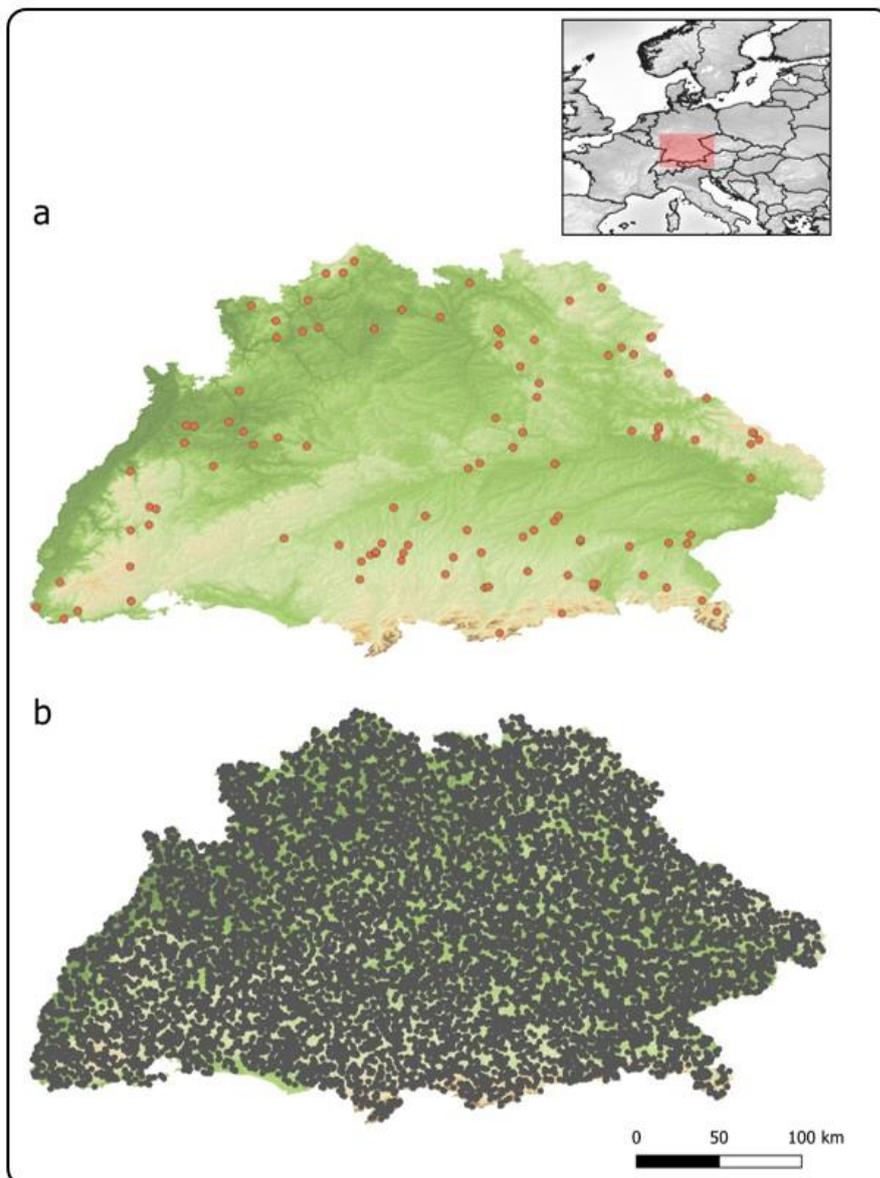
Figure S2 Map of the aquaculture facilities (a; N = 107) and background samples (b; N = 10102) used for training of the final MaxEnt model to predict environmental suitability for salmonid aquaculture in southern Germany. Red shade in top right panel illustrates the extent of the study area within Europe.

**Table TS2** Predictor variables of environmental suitability for salmonid aquaculture in southern Germany used in the MaxEnt model, including justification for inclusion and data source.

Variable	Justification	Source
Isothermality (bio3), max temperature of warmest month (bio5), min temperature of coldest month (bio6)	Temperature is known to affect physiology and life history of fish. Data are for air temperature, which is assumed to correlate with water temperature in flow-through aquaculture systems.	Worldclim.org*
Precipitation of wettest month (bio13), precipitation seasonality (bio15)	Precipitation during the wettest month can be considered as a surrogate predictor of flood risk, while precipitation seasonality can indicate seasonal variation in water supply/inflow discharge. Both aspects are assumed to affect environmental suitability for flow-through aquaculture operations.	Worldclim.org*
Roughness, northness	Terrain roughness was used as surrogate predictor of stream flow, which directly relates to water supply for aquaculture facilities. Roughness may further affect the layout of raceways in flow-through systems. Northness describes the terrain orientation. Value close to 1 corresponds to a northern exposition on a vertical slope, i.e., a very low amount of solar radiation, while a value close to -1 indicates a very steep southern slope, exposed to a high amount of solar radiation. Solar radiation may affect temperature regimes in aquaculture.	Earthenv.org
Human Footprint	The Human Footprint is a composite measure of human infrastructure and population, built-up environments, and agriculture, all of which may affect suitability for aquaculture operations, e.g., by providing road and energy access or nearby homes for workers.	Socioeconomic Data and Applications Center (SEDAC)
Hydrological substrate	The hydrological substrate describes four levels of surface geology with different aquifer properties. These properties may affect groundwater availability and spring discharge, which is relevant for flow-through aquaculture operations.	Bundesanstalt für Geowissenschaften und Rohstoffe (German Federal Institute for Geosciences and Natural Resources).

*: Climate data were obtained for near-current (1970-2000) and projected end-of-the-century (2081-2100) conditions across four ssps (ssp126, ssp245, ssp370 and ssp585) based on the MPI-ESM1-2-HR model.
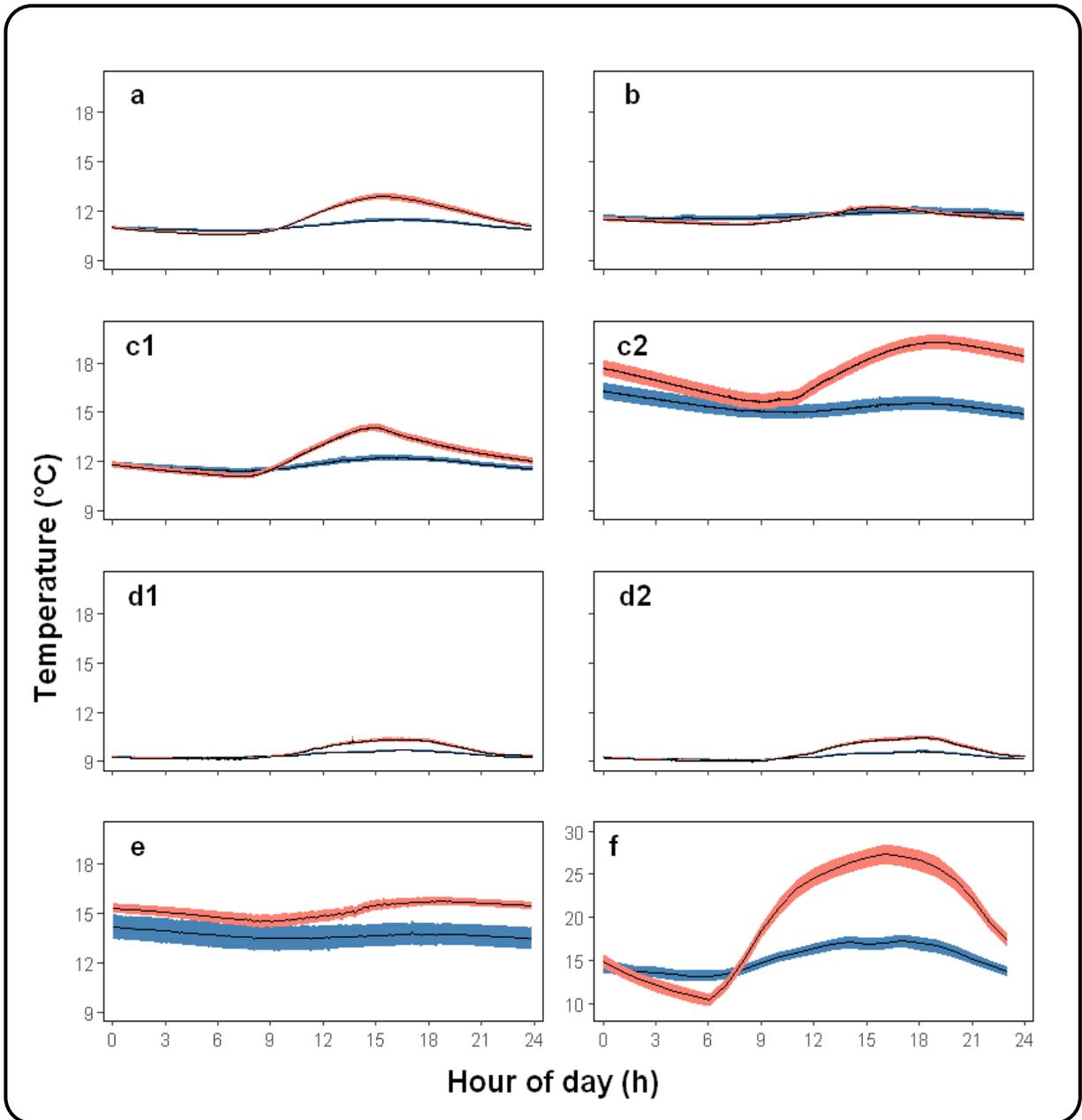
Figure S3 Mean daily variation from July to August at different study sites. Sunny days (red) vs cloudy days (blue). Letters a – e according to study site; f = mean ambient temperature; c1 / d1 = first pond in row; c2 / d2 = last pond in row.

Table TS3 ARIMA models for the test of effects of potential drivers of temperature increase on daily basis. ARIMA Model (2,0,2) with non zero mean. Correlation of the observed variables with the measured water temperature data; z-test of coefficients. * = <0.05; ** = <0.01; *** = <0.001; ar1, ar2: auto regression coefficients; ma1, ma2: moving average coefficients; S1 = sun impact day before (Lead = 1); Ta = ambient temperature; P1 = Rainfall day before (lead = 1); Loc. A – E = Dummy variables for different localities.

Variable	Estimate	Std. Error	z value	Pr(>|z|)	Significance
ar1	1.47	0.03	48.75	< 0.001	***
ar2	-0.47	0.03	-15.88	< 0.001	***
ma1	-0.53	0.03	-17.82	< 0.001	***
ma2	-0.20	0.01	-14.15	< 0.001	***
LocA	10.06	0.44	22.64	< 0.001	***
LocB	9.41	0.50	18.65	< 0.001	***
LocC	9.54	0.45	21.01	< 0.001	***
LocD	8.87	0.51	17.46	< 0.001	***
LocE	9.05	0.61	14.83	< 0.001	***
P1Ta	0.12	0.04	3.04	< 0.01	**
S1P1	0.24	0.04	5.68	< 0.001	***
S1Ta	0.00	0.00	1.19	0.24	
P1LocA	-1.58	0.56	-2.81	< 0.01	**
S1LocA	-0.01	0.00	-2.10	0.04	*
TaLocA	0.08	0.00	20.36	< 0.001	***
TaLocB	0.07	0.00	17.73	< 0.001	***
P1LocB	-2.05	0.57	-3.61	< 0.001	***
S1LoCB	0.00	0.00	0.79	0.43	
P1LocC	-3.66	0.79	-4.62	< 0.001	***
S1LocC	0.04	0.00	14.91	< 0.001	***
TaLocC	0.19	0.01	31.83	< 0.001	***
P1LocD	-1.81	0.56	-3.23	< 0.01	**
S1LocD	-0.01	0.00	-2.75	< 0.01	**
TalocD	0.04	0.00	9.13	< 0.001	***
P1LocE	-1.58	0.73	-2.18	0.03	*
S1LocE	0.00	0.00	1.67	0.10	.
TalocE	0.14	0.00	31.55	< 0.001	***

Table TS4 Outcome of the monthly linear model used for backward and future projection of water temperatures. Significance codes: '***' <0.001 '**' <0.01 '*' <0.05 '.' <0.1. Tamin / Tamax = minimum and maximum ambient temperature (° C); P = rainfall (mm / 5 min); Loc.A – Loc.C are dummy variables for the different localities (Loc.B is the reference location of the standard linear model treatment contrast).

Variable	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	9.11	0.53	17.24	< 2e-16	***
Loc.A	0.25	0.68	0.37	0.71	
Loc.C	-6.00	1.12	-5.34	0.00	***
Loc.D	-0.10	0.68	-0.14	0.89	
Loc.E	-1.35	0.73	-1.84	0.07	.
Tamin	0.22	0.07	3.03	0.00	**
Tamax	0.02	0.05	0.34	0.74	
P	-0.01	0.00	-2.24	0.03	*
Loc.A:Tamin	-0.11	0.08	-1.36	0.18	
Loc.C:Tamin	-0.22	0.11	-2.01	0.05	*
Loc.D:Tamin	-0.19	0.09	-2.21	0.03	*
Loc.E:Tamin	0.11	0.09	1.18	0.24	
Loc.A:Tamax	0.00	0.06	0.02	0.99	
Loc.C:Tamax	0.51	0.09	5.96	0.00	***
Loc.D:Tamax	-0.02	0.06	-0.30	0.77	
Loc.E:Tamax	0.10	0.06	1.60	0.11	
Loc.A:P	0.00	0.00	1.57	0.12	
Loc.C:P	0.01	0.00	1.58	0.12	
Loc.D:P	0.00	0.00	1.02	0.31	
Loc.E:P	0.00	0.00	1.01	0.32	
Tamin:Tamax	0.00	0.00	0.41	0.69	
Tamin:P	0.00	0.00	-0.68	0.50	
Tamax:N	0.00	0.00	1.13	0.26	
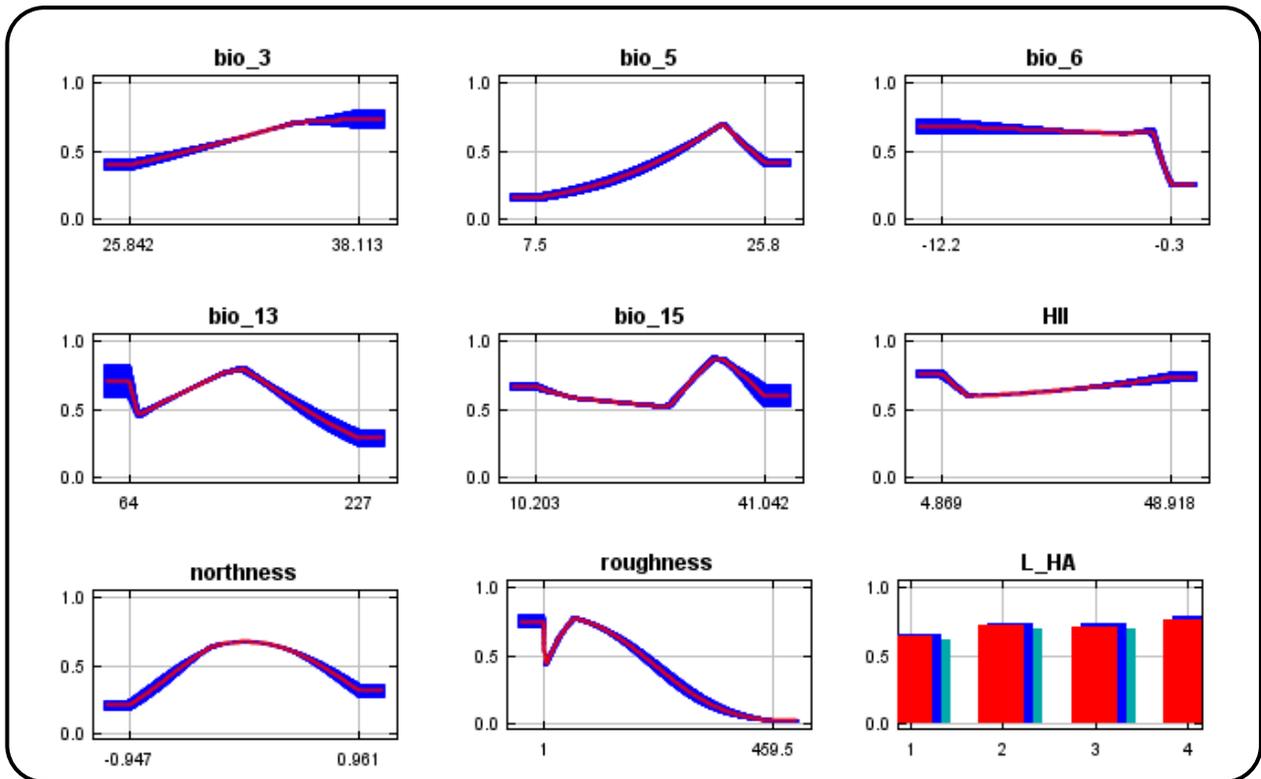
Figure S4 Response curves of environmental variables in MaxEnt models used to predict environmental suitability for salmonid aquaculture in southern Germany, showing mean response across 20 replicate runs with cross-validation (red) ± standard deviation (blue, two shades for categorical variables). Abbreviations for bioclimatic variables are as follows: bio_3 – isothermality, bio_5 - max temperature of warmest month, bio_6 - min temperature of coldest month, bio_13 - precipitation of wettest month, and bio_15 - precipitation seasonality. Level codes for hydrological substrate (L_HA) are 1 - "pore aquifers", 2 - "combined pore and fractured aquifers", 3 - "fractured and karst aquifers" and 4 - "aquitards and aquicludes". HII indicates the Human Footprint.

Table TS5 Comparison of maximum mean temperatures (July and August) and yearly mean temperature. $\Delta T_{S/C}$ = temperature difference on sunny/cloudy days. $\Delta TMin/Max$ = temperature minimum/maximum on sunny (s) or cloudy (c) days. Temperatures were measured at two different sites in the runways: 1 = at the start of the runway: 2 at the end of the runway.

Study site	N_C	$TMin_C$ [°C]	$TMax_C$ [°C]	ΔT_C [°C]	N_S	$TMin_S$ [°C]	$TMax_S$ [°C]	ΔT_S [°C]
B1	57	11.40 ± 0.38	12.34 ± 1.05	0.94 ± 0.91	45	11.16 ± 0.34	12.25 ± 0.4	1.09 ± 0.15
B2	54	11.46 ± 0.36	12.63 ± 0.93	1.17 ± 0.79	42	11.15 ± 0.4	13.15 ± 0.81	2 ± 0.66
C1	63	11.30 ± 0.60	12.48 ± 0.85	1.18 ± 0.64	43	11.08 ± 0.57	14.13 ± 0.76	3.06 ± 0.38
C2	80	14.54 ± 1.59	16.6 ± 2.1	2.07 ± 1.28	49	15.59 ± 1.55	19.32 ± 1.43	3.73 ± 0.56
D1	63	9.15 ± 0.18	9.76 ± 0.31	0.6 ± 0.26	43	9.1 ± 0.17	10.55 ± 1.12	1.45 ± 1.1
D2	80	9.03 ± 0.18	9.66 ± 0.35	0.63 ± 0.26	49	8.97 ± 0.16	10.56 ± 0.62	1.6 ± 0.53

Table TS6 Calculated changes in ammonia and carbon dioxide concentrations in dependence on percentage of water availability. Values refer to daily amounts of ammonia/carbon dioxide. Red values indicate harmful concentrations.

Scenario	Unit	200%	100%	75%	50%	25%
Flow rate	L/s	120	60	45	30	15
Raceway volume	L	300	300	300	300	300
Ammonia	mg/L	0.005	0.010	0.013	0.02	0.04
CO_2	mg/L	10	20	26.67	40	80
Ammonia increase	%	-50	0	+33	+100	+300
CO_2 increase	%	-50	0	+33	+100	+300

Formulas for TS6:

To calculate the increase in NH_3 and CO_2 with a decreased water inflow, first the daily water volume (DWV) was calculated for days with either 100%. 200%. 75%. 50% or 25% of water inflow:

$$DWVx[L] = \left(Flow\ rate\ \left[\frac{L}{s}\right] * 60 * 60 * 24\right) * x + raceway\ volume$$

With x being a free variable for the percentage of water flow.

Afterwards, the daily amount of NH_3 (DNH_3) respectively CO_2 (DCO_2) within the system was calculated for the 100% DWV scenario, representing the normal state:

$$DNH_3 = Ammonia_{100}\ \left[\frac{mg}{L}\right] * DWV_{100}\ [L]$$

$$DCO_2 = CO_{2\ 100}\ \left[\frac{mg}{L}\right] * DWV_{100}\ [L]$$

With $Ammonia_{100}$ and $CO_{2\ 100}$ being realistic example values.

The total amount of both ammonia and carbon dioxide in correlation to water availability was than calculated with

$$Ammonia_y\ \left[\frac{mg}{L}\right] = \frac{DNH_3}{DWV_x}$$

$$CO_{2y}\ \left[\frac{mg}{L}\right] = \frac{DCO_2}{DWV_x}$$

With DWV changing in percentage of water availability (200. 75. 50. 25 %) and $Ammonia_y$/CO_{2y} being the related ammonia / CO_2 concentration.

At the end, increase in percentage of NH_3 and CO_2 was calculated

$$[NH_3]_{change}\ [\%] = \frac{Ammonia_y}{Ammonia_{100}} * 100$$

$$[CO_2]_{change}\ [\%] = \frac{CO_{2\ y}}{CO_{2\ 100}} * 100$$

3. Life cycle assessment of rainbow trout farming in the temperate climate zone based on the typical farm concept, a benchmark of region-level assessment

3.1. Abstract

Fish from aquaculture has the ability to meet consumer demand for a healthy and sustainable diet. The environmental footprint of fish farming depends largely on the method of production. Trout production in the temperate climate zone in open flow-through systems using the natural hydraulic gradient of streams as freshwater supply is energy efficient relative to systems that are more intensive. To investigate the possibility of reducing global warming potential, eutrophication potential, acidification potential, and ozone layer depletion associated with rainbow trout (*Oncorhynchus mykiss*) production in southern Germany, a comprehensive dataset collected under the typical farm framework was analyzed and a cradle-to-gate life cycle assessment was conducted. The impact of feed source (plant- and fish-based) was analyzed, as well as that of photovoltaic panels installed over the production area. Calculated emissions per kg fish live weight were 1.18 kg CO_{2eq}, 7.89e-8 kg CFC_{11eq}, 0.00552 kg SO_{2eq}, and 0.0257 kg PO_{4eq}. Full covering of the production area with photovoltaic panels, compared to the current ~40 % coverage, was estimated to provide a reduction of 1.04 kg CO_{2eq}, resulting in emissions of 0.773 kg CO_{2eq} per kg fish live weight. Feeds containing 35 % and 61.8 % fish meal were associated with lower emissions compared to 100 % plant-based, with a reduction in global warming potential (GWP) of 0.79 kg CO_{2eq} in the 61.8 % fish meal variant. Results showed that the use of photovoltaic panels can significantly reduce rainbow trout culture impact on the analyzed environmental impact categories. Feed containing fish meal has a lower impact on the analyzed environmental impact categories. Alternatives such as insect meal and sustainable plant alternatives should be the focus of future research.

3.2. Introduction

Agricultural production of food for human consumption contributes for 17 % of global greenhouse gas (GHG) emissions (FAO, 2020). Consequently, diet choices can play an important role in their reduction and those of other environmental emissions (Rabès et al., 2020; Scarborough et al., 2014).

The cultivation of plants for food products has relatively low environmental impact and is considered more sustainable than animal products (Chai et al., 2019; Pimentel and Pimentel, 2003), although processed plant-based foods can have high environmental impact, especially relative to their nutritional content (Berardy et al., 2020; Clune et al., 2017; Smedman et al., 2010). The environmental impact of farmed animals is often much greater than that of plants but varies depending on species and type of farming (Clune et al., 2017; Eriksson et al., 2005; Leinonen and Kyriazakis, 2016; Lynch, 2019; Samuel-Fitwi et al., 2013a).

Studies have shown that aquaculture has a relatively low ecological impact (Bava et al., 2017; d'Orbcastel et al., 2009; Desjardins et al., 2012; Lynch, 2019; Samuel-Fitwi et al., 2013a). Reasons for this include the adaptation of fish to an aquatic environment. Most fish are neutrally buoyant in the water column and do not need a strong skeletal structure, which results in a low bone to flesh ratio and high edible yield (Brinker and FFS Langenargen, 2019; Hoha et al., 2013). As poikilotherms, fish do not invest energy in maintaining body temperature and are efficient feed converters when reared at the appropriate temperature range (Costa-Pierce et al., 2012; Ebeling and Timmons, 2010; Wedemeyer, 1996).

The nutritional value of a food product must be considered when evaluating its environmental costs. Fish possess a variety of valuable and essential nutrients including Omega-3 fatty acids, vitamins, selenium, and iodine (Tacon et al., 2020). With the exception of chicken, fish is considered the only farmed vertebrate, that in relation to living weight or edible product, provides not only essential micro-nutrients but other documented health benefits as well (Clark et al., 2019). Salmonids such as trout show a particularly low environmental impact among most species of farmed fish (Grönroos et al., 2006; Samuel-Fitwi et al., 2013a).

Approximately one-third of the salmonid output of Germany is produced in the federal state of Baden-Wuerttemberg, predominately in open flow-through systems (Destatis, 2021). These farming systems have a low energy demand as they chiefly use fresh water from a river or well, which flows continuously through the system by using the natural hydraulic gradient (Fornshell et al., 2012). Such open systems have the potential to emit nutrients into the receiving water (d'Orbcastel et al., 2009; Philis et al., 2019; Samuel-Fitwi et al., 2013a). There is a growing trend towards partially or fully closed recirculating aquaculture systems (RAS) in which a significant proportion of water is mechanically and biologically filtered before reuse for production. This results in an

increased energy demand by pumping and water treatment. Conversely, waste water treatment is more efficient since the wastewater stream is concentrated and channeled for treatment (Ebeling and Timmons, 2012, 2010). As a consequence, the RAS is less likely to emit nutrients into the adjacent aquatic environment (d'Orbcastel et al., 2009; Philis et al., 2019; Samuel-Fitwi et al., 2013a).

To quantify and compare the total environmental impact of a product a standard benchmark including all relevant steps involved in the production cycle is required. Life cycle assessment (LCA) is an effective tool for generating detailed and integrative information with respect to impact categories like global warming potential (GWP), ozone layer depletion (ODP), and eutrophication potential (EP) of a given product within defined boundaries (Martins et al., 2010). It allows identifying impact peaks in the production chain as well as detecting areas for improvement.

Life cycle assessments in agriculture are commonly based on data collected from individual representative farms, which, especially in inland aquaculture, are characterized by unique conditions (Ridder, 2017). The *typical farm approach* is an Agri Benchmark Network analysis element that compensates for differences among farms (Lasner, 2020) providing the opportunity to collect, interpret, and generalize viable farm characteristics in close exchange with farmers (Chibanda et al., 2020; "Fish - agri benchmark," 2020; Lasner, 2020). Conditions can vary with the framework, showing the impact of multiple variables on an individual farm (Lasner, 2020). For details of the typical farm approach see (Chibanda et al., 2020; Lasner, 2020).

As every sector, aquaculture is constantly evolving. One recent innovation trend is the utilization of roofing over farms which is used to shade the water and reduce its temperature. The roof surface can additionally be used for installation of solar panels to produce photovoltaic energy, reducing the environmental impact of a farm.

Another evolving aspect of the aquaculture sector is the improvement of fish feed with the aim of reducing its potential for environmental degradation (Bohnes et al., 2019). The efforts to make fish feed more sustainable have initiated a trend of substituting fish meal with plant ingredients in aquafeeds (Bartley et al., 2007; d'Orbcastel et al., 2009; Martins et al., 2010).

The aim of present study was to evaluate the capacity for reduction in negative environmental effects of trout culture. Several environmental impact categories were analyzed with respect to feed composition and energy use.

3.3. Material and Methods

Goal and scope. The purpose of this research was to estimate selected categories of environmental impact associated with the production of 1 kg live weight (LW) rainbow trout in a traditional central European salmonid flow-through culture system. Impact categories were evaluated with respect to feed composition and energy use. Production processes and materials used were considered, with regard to their energy and material flows.

System description. A large proportion of data used in these analyses was collected in 2013 from a trout farm operating at three locations in separate river systems in Baden-Wuerttemberg, southern Germany. Information on land, energy, feed and oxygen use has not changed since assessment period. Also no additional vehicles were purchased on-farm. Meanwhile, energy composition changed slightly and has therefore been updated accordingly to assure timeliness of data. Rainbow trout *Oncorhynchus mykiss* are reared from egg to market size, with an annual production of ~500 tons. Feed was composed of 35 % fish meal and 15 % fish oil and therefore close to the feed composition given in Brinker and Reiter (2011). As a consequence, the feed composition of the feed used was modelled accordingly. The economic feed conversion ratio (Table 6) of 0.89 is similar to the FCR given in Brinker and Reiter (2011).
Each kg of trout produced requires 0.9 m² of land. Water entering the raceways originates from upper reaches of small streams. The farm uses common monitoring systems to control the water quality. Water is treated at the terminus of the raceways with a drum filter to remove solids and / or held in maturation / sedimentation ponds. Approximately 40 % of the fish production area (raceways for grow out) is currently roofed and used for a photovoltaic system (low voltage, 3 kWp with a slanted roof installation and single SI panels) that provides a renewable energy source for farming operations.

Life cycle assessment and data collection. The LCA was conducted following the ISO standards 14040 and 14044 (Deutsches Institut für Normung e.V., 2006; Finkbeiner et al., 2006; Lee and Inaba, 2004). A cradle to farm gate assessment was based on data collected principally under the typical farm approach according to conditions described by Lasner (2020). The observed farm needs to be "long-established, economically stable, well equipped and well managed, but not too far ahead of their competitors." It is essential that detailed data of the farm operation be available (Lasner, 2020).

41

Data was collected by reviewing the economic accounts and processed according to the typical farm approach of the year 2013. A cradle-to-gate assessment was accomplished in two steps: First, a real concept representative farm model was used to assess current emissions. Second, a modal-real concept method was used to investigate alternative production scenarios with a photovoltaic system on a shaded area of the plant site and a reduction of fish meal in feed (Lasner, 2020). Information of feed formulation as published in Brinker and Reiter (2011) was obtained from the supplier and goes beyond the publicly accessible information of feed composition. Nitrogen and phosphorous emissions originating from faeces, uneaten feed and metabolic end products were calculated as a mean value. This mean value was based on volume of emissions per kg feed or emissions detected in various studies on this topic (Axler et al., 1997; Bureau et al., 2003; Chatvijitkul et al., 2017; d'Orbcastel et al., 2008; Dekamin et al., 2015; Elhami et al., 2019; Pulatsu et al., 2004).

The software program SimaPro 9.1.1.1 was used to assess the relevant ecological impact categories of rainbow trout production, with the databases Ecoinvent v. 3.6, Agri-footprint v. 5.0, and Agribalyse v. 3.0. The CML-IA baseline/EU 25 method was used for calculations of the environmental impact. CML was chosen to guarantee comparability with other studies on animal farming and food production using mainly CML (Ayer and Tyedmers, 2009; Dekamin et al., 2015; Mungkung et al., 2013; Philis et al., 2019; Samuel-Fitwi et al., 2013a; Yacout et al., 2016). To test for uncertainties, a Monte-Carlo simulation was run in SimaPro with 5000 runs to the 95th confidence interval. Uncertainty analysis was performed on the systems environmental performance.

Impact categories. The assessed impact categories were selected for their direct effect on the environment and biodiversity (GWP, AP, EP), as well as on human health (ODP). The categories span a broad range of effects related to feed and energy use in aquaculture (Acero et al., 2016; Anwar et al., 2016; Aubin et al., 2009; Cao et al., 2013). The Global warming potential (GWP) is a result of anthropogenic activity and natural processes. Gases like methane and nitrous oxides can accumulate in the atmosphere and contribute to climate change and biodiversity loss (Acero et al., 2016; Cao et al., 2013). The various gases are converted into CO_2 equivalents, depending on their relative impact compared to CO_2. The GWP of the evaluated factors is calculated for a 100-year period (Acero et al., 2016; Myhre et al., 2013).

Another impact factor observed is the Ozone layer depletion (ODP), which is given in chlorofluorocarbon equivalent (CFC_{11eq}). It is affected by halons or hydro-chlorofluoro-carbons and represents the contribution of the analyzed factors to the destruction of the ozone layer, which results in increased UV-radiation, affecting ecosystems as well as increasing the risk of disorders including skin cancer and cataracts in humans (Acero et al., 2016; Anwar et al., 2016).

Additionally, the acidification potential, which is given in SO_{2eq} was analyzed, as well. Due to emissions like SO_x, NO_x, and NH_3 the pH of soil and water decrease. The resulting acidification can affect entire ecosystems (Acero et al., 2016).

The eutrophication potential (EP), which is critical to evaluating the effect of production processes on adjacent aquatic systems. The observed nutrients, mainly nitrogen and phosphorus, increase the productivity of a system to a point above sustainable growth, leading to reduced water quality. The EP is given in kg PO_{4eq}. (Acero et al., 2016).

System boundaries overview. Seven primary processes involved in the production of rainbow trout from fry (15 g) to plate size (380 g) were divided into several sub-processes (Figure 6). Trout culture input includes feed, its production and transport, area of land-use, on-farm vehicles and their fuel use including background processes of fuel production, liquid oxygen produced via cryogenic air separation with its production cycle and transport, and electricity used for mechanical devices such as those used for water quality control and automated feeding. A mean value of phosphorous and nitrogen discharge into the water was calculated according to other research (Axler et al., 1997; Bureau et al., 2003; Chatvijitkul et al., 2017; d'Orbcastel et al., 2008; Davis, 2015; Dekamin et al., 2015; Elhami et al., 2019; Pulatsu et al., 2004).

Water use was not considered since, in a flow-through system, water is returned to the stream from which it is sourced. Effluent treatment usually ensures that the outflowing water does not contain a substantially greater quantity of nutrients or pollutants than the inflow (Fornshell et al., 2012).

The LCA of the model farm was based on limited generation of photovoltaic power and feed with 35 % fish meal content. The FCR was 0.89 according to the farmer's calculation. The diet composition was able to compete with commercially available feed (Brinker and Reiter, 2011). All analyzed factors were included in the calculations (Figure 6).
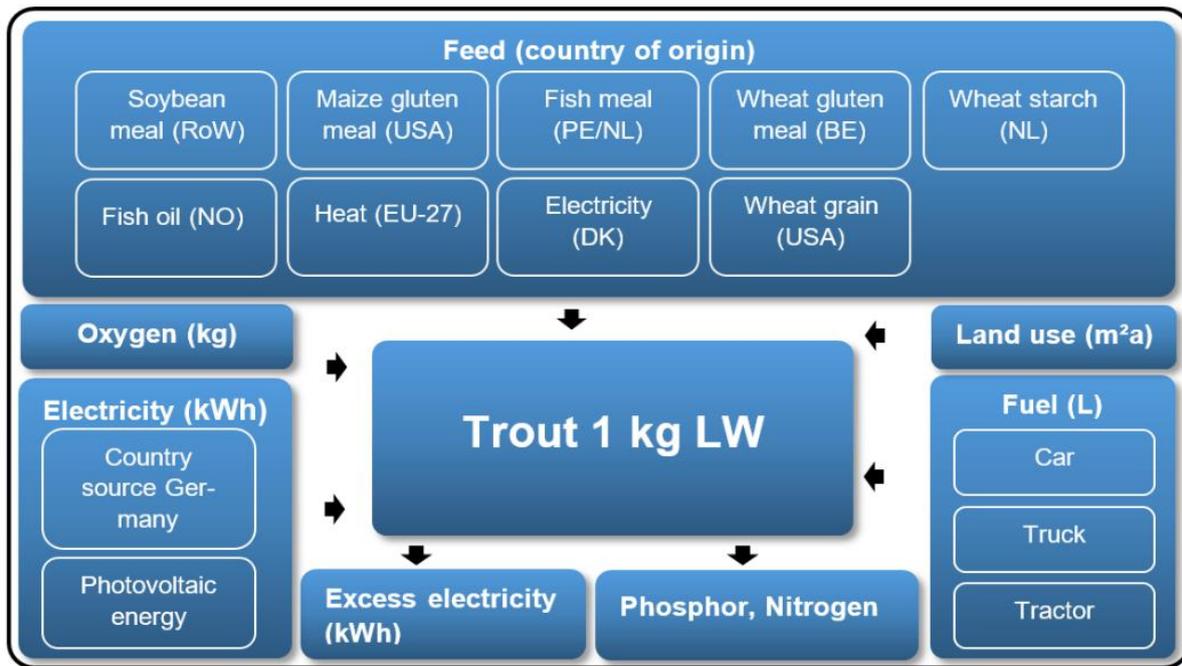
Figure 6 Energy flow associated with production of 1 kg LW rainbow trout in a model salmonid farm. Excess electricity is produced by photovoltaic panels. Heat in feed production is needed for pellet extrusion. RoW = Rest of World; tkm = ton-kilometers; m²a = m² per year; NO = Norway; PE = Peru; NL = Netherlands; BE = Belgium; USA = United States of America; DK = Denmark; EU27 = Europe.

Life cycle inventory - Solar cells and electricity source. Data of electricity was obtained from EcoInvent 3.1. Since the database provides only the current composition of electricity in Germany, it deviates to some extent from that in 2013. Hence, the current common German energy mix of 50 % fossil energy and 50 % renewable energy was used in this analysis. The mining, transport and infrastructure, energy, and water involved in the energy production systems was considered by the software as given in the databases, additionally, the manufacture of the photovoltaic system and mounting of the photovoltaic panels were factored in. Regarding the photovoltaic system, life span and maintenance are considered. To assure comparability between German energy mix and electricity produced by the photovoltaic panels, the photovoltaic system is considered new, since energy mix was adjusted according to the current energy mix, as described earlier.

Life cycle inventory - Fish feed. Feed production included the steps in the production of individual ingredients: planting, fertilization, pesticide and weed treatment, and harvest of plant-based components; catching and processing fish for fish meal and oil; transportation; and the extrusion process. The three feed formulations were modelled using data from Agribalyse 3.0 and Agri-footprint 5.0 according to Brinker and Reiter (2011), with differing fish meal content (Table 5). Data of mineral and vitamin

content were derived from the Agribalyse-database, while transport data originated from EcoInvent 3.6. For the principal feed ingredients, all relevant databases were used and origin and quantity of ingredients were considered as far as possible.

Table 5 Fish feed composition derived from Brinker and Reiter (2011) in percentage. FM = fish meal; NO = Norway; PE = Peru; NL = Netherlands; RoW = Rest of World; BE = Belgium; USA = United States of America.

Component (origin)	No FM	35 % FM	61.8 % FM
Fish oil (NO)	17.15	15.55	14.155
Fish meal (PE/NL)	-	35	61.8
Soybean meal (RoW)	35.1	17.5	-
Wheat gluten meal (BE)	25.3	14.2	-
Wheat grain (USA)	10	10	10
Wheat starch (NL)	6.1	7.3	13.6
Maize gluten meal (USA)	2.5	-	-
Vitamins and minerals[1]	3.805	0.45	0.445

Composition adjusted for vegetable diet by (Brinker and Reiter, 2011) according to NRC (1993) to have a comparable nutritional value of the different feeds.

Scenario analysis. In analyses of energy use, GWP, ODP, AP, and EP were estimated considering full coverage of production area with roofs and photovoltaic panels (100 % PV) compared to a scenario without photovoltaic energy (No PV) and to the model farm state of partial coverage (~40 % PV) with roof equipped with photovoltaic panels. All photovoltaic scenarios were calculated with the use of 35 % fish meal feed (Supplements, Table TS9).

In the No PV scenario, no energy was added to the net energy of the grid. The surplus electric energy in the 40 % PV and the 100 % PV scenario was fed into the power grid. To calculate the energy production of the photovoltaic system an extrapolation with respect to panel surface and kWp was done. From this value the needed kWh on farm was subtracted to receive the amount of surplus energy. Electricity output was considered as German medium voltage mix in the software. Excess electric energy from photovoltaic generation is categorized as *output* electricity, while *input* represents electricity needed for the production of 1 kg LW rainbow trout (Table 6).

In the second analysis, the production of 1 kg trout was compared under three feed formulations (Table 5): high fish meal content (61.8 % FM), medium fish meal (35 % FM), and without fish meal (No FM). The FCRs were as determined by Brinker and Reiter (2011), 0.97 for No FM, 0.89 for 35 % FM equivalent to that on the

model farm, and 0.81 for 61.8 % FM (Table 6,Supplements, Table TS7) (Brinker and Reiter, 2011).

Table 6 Input and output associated with the production of 1 kg rainbow trout. The feed composition scenarios were modelled with ~40 % photovoltaic coverage, while the different photovoltaic scenarios were modelled with the 35 % fish meal feed. Model farm values = 35 % FM and ~40 % PV. m²a = m² per year land use; p = parts; tkm = ton-kilometer; FM = fish meal; PV = photovoltaic.

Input	Unit	35 % FM	No FM	61.8 % FM
Land use	m²a	0.09	0.09	0.09
Feed	kg	0.89	0.97	0.81
Vehicles	p	0.000016	0.000016	0.000016
Fuel	L	0.01	0.01	0.01
Oxygen	kg	0.67	0.67	0.67
Electricity	kWh	0.16	0.16	0.16
Output	**Unit**	**~40 % PV**	**No PV**	**100 % PV**
Rainbow trout	kg	1	1	1
Electricity	kWh	0.94	0	1.65
Phosphorous	kg	0.01	0.01	0.01
Nitrogen	kg	0.06	0.06	0.06

3.4. Results

Model farm. The GWP of 1 kg LW rainbow trout produced under the typical farm conditions (Photovoltaic of 40 % and a diet with 35 % fish meal) was calculated to be 1.18 kg CO_{2eq}; ODP was $7.89e^{-8}$ kg CFC_{11eq}; AP was 0.00552 kg SO_{2eq}; and EP was 0.0257 kg PO_{4eq}. While feed and technical oxygen contributed the largest share of the observed impact categories, electricity added relatively little to total emissions (Figure 7). More detailed information on various impacts can be found in the 3.7 Supplements, Table TS10.

Photovoltaic electricity generation. Comparative analysis of energy use showed No PV to have the highest environmental impact in three of four analyzed categories (Table 7). With a roof and solar panel coverage of approximately 40 % of the production area, GWP and ODP can be reduced by 35 % compared to No PV scenario, while AP is reduced by nearly 20 % and EP by 9 %. Furthermore, results showed that the scenario with 100 % PV coverage can reduce GWP by more than 57 %, ODP by ~50 %, and AP by 32 %. Eutrophication potential is reduced to 84 % of the No PV scenario value (Figure 8).
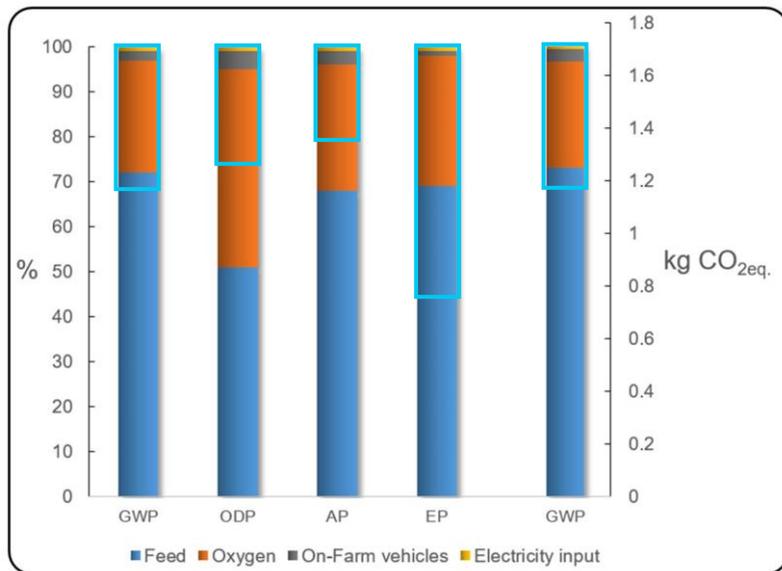
Figure 7 Relative contribution of the analyzed processes of the typical farm to GWP, ODP, AP, and EP associated with production of 1 kg LW rainbow trout. Absolute contribution of production processes to GWP is shown. Emissions counteracted by excess electric energy production (current state with 40% photovoltaic covering) are marked by blue frames. GWP = global warming potential; ODP = ozone layer depletion; EP = eutrophication potential; AP = acidification potential.
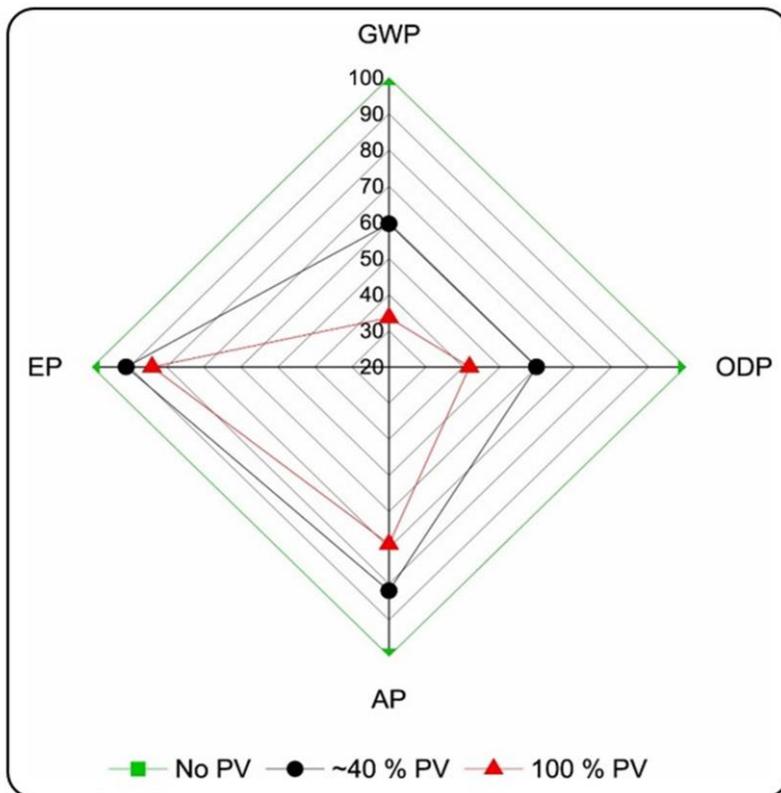
Figure 8 Contribution of trout culture to selected environmental impact categories with respect to proportion of system covered with photovoltaic panels.
PV = photovoltaic energy;
GWP = global warming potential;
ODP = ozone layer depletion;
EP = eutrophication potential;
AP = acidification potential.

Table 7 Impact of 1 kg LW rainbow trout production with different levels of on-site photovoltaic power generation on several environmental categories. PV = photovoltaic energy; GWP = global warming potential; ODP = ozone layer depletion; EP = eutrophication potential; AP = acidification potential.

Impact categories	GWP (kg CO_{2eq})	ODP (kg CFC_{11eq})	AP (kg SO_{2eq})	EP (kg PO_{4eq})
No PV	1.81	$1.23e^{-7}$	0.0068	0.0282
~40 % PV	1.18	$7.89e^{-8}$	0.0055	0.0257
100 % PV	0.77	$5.92e^{-8}$	0.0046	0.0238

By replacing German electricity mix with on-site photovoltaic generation, a reduction of nearly 0.25 kg CO_{2eq} per kg LW trout is achieved. At the same time, the excess electric energy produced by the 40 % coverage saves 0.63 kg CO_{2eq}. The 100 % coverage would increase savings to even more: 1.037 kg CO_{2eq} per kg trout (Figure 9).
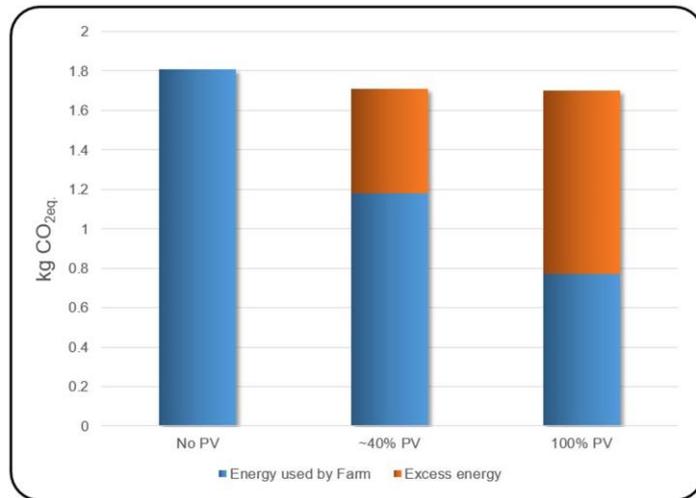
Figure 9 Global warming potential as CO_{2eq} associated with production of 1 kg LW rainbow trout and estimated savings in CO_{2eq} by photovoltaic energy (PV).

Feed fish meal content. Table 8 presents the total GHG emissions calculated for the analyzed feed formulations, depending on their different FCRs. Lowest emissions were found for the formulation with 61.8 % fish meal, while the formulation without fish meal resulted in the highest emissions. Based on the LCA, the 61.8 % fish meal variant is superior with respect to all measured parameters. Its use has the potential to reduce GWP by 0.79 kg CO_{2eq} and ODP by $4.4e^{-8}$ kg CFC_{11eq} compared to the no fish meal variant.

In terms of percentages, it produces 50 % less CO_2 and 40 % less CFC_{11eq} emissions compared to feed containing no fish meal. Nevertheless, a fish meal content of 35 % can already reduce GWP by 30 % and ODP by 25 % compared to a plant-based fish meal feed. Both formulations containing fish meal showed 5–10 % lower AP and EP than the feed without fish meal (Figure 10a). The differences in environmental impact of the feed formulations are chiefly related to their ingredients. Compared with the formulation containing no fish meal, the 61.8 % fish meal feed produced significantly lower GWP (~35 %) and ODP (~50 %). Eutrophication potential is nearly 45 % lower in processing the high fish meal diet compared to the no fish meal diet. The AP of the 100 % plant-based diet is 4 % lower than that of the 61.8 % fish meal feed, although the 35 % fish meal feed has the lowest AP being 7 % lower than that of the 61.8 % fish meal feed (Table 8).

A closer inspection of the emissions of the different feed components revealed that wheat, soy, and fish meal make the largest contribution to environmental impact (Figure 10b). Regarding the No FM feed especially wheat contributes largely to emissions being responsible for more than 50 % of the AP and EP and 38 % of the ODP. In the 35 % FM variant, wheat contributed 35 % of AP and ODP, while EP in the 35 % and 61.8 % fish meal feed is affected by ~65 % by the addition of wheat in the feed. The main share of the GWP can be traced back to soy which was responsible for 54 % in the no fish meal formulation and 34 % in the 35 % FM variant. Fish meal is the source of 55 % of AP and GWP of the 61.8 % fish meal variant, while transport exerts the largest effect on ODP (44 %). For more information, see Supplements, Table TS10.

Table 8 Selected categories of environmental impact associated with the production of 1 kg LW rainbow trout relative to feed formulation and FCR. GWP = global warming potential; ODP = ozone layer depletion; EP = eutrophication potential; AP = acidification potential; FM = fish meal; FCR = feed conversion ratio.

Impact categories Feed variant	FCR	GWP (kg CO_{2eq})	ODP (kg CFC_{11eq})	AP (kg SO_{2eq})	EP (kg PO_{4eq})
No FM	0.97	1.63	$1.06e^{-7}$	0.0061	0.0272
35 % FM	0.89	1.18	$7.89e^{-8}$	0.0055	0.0257
61.8 % FM	0.81	0.84	$6.19e^{-8}$	0.0054	0.0248
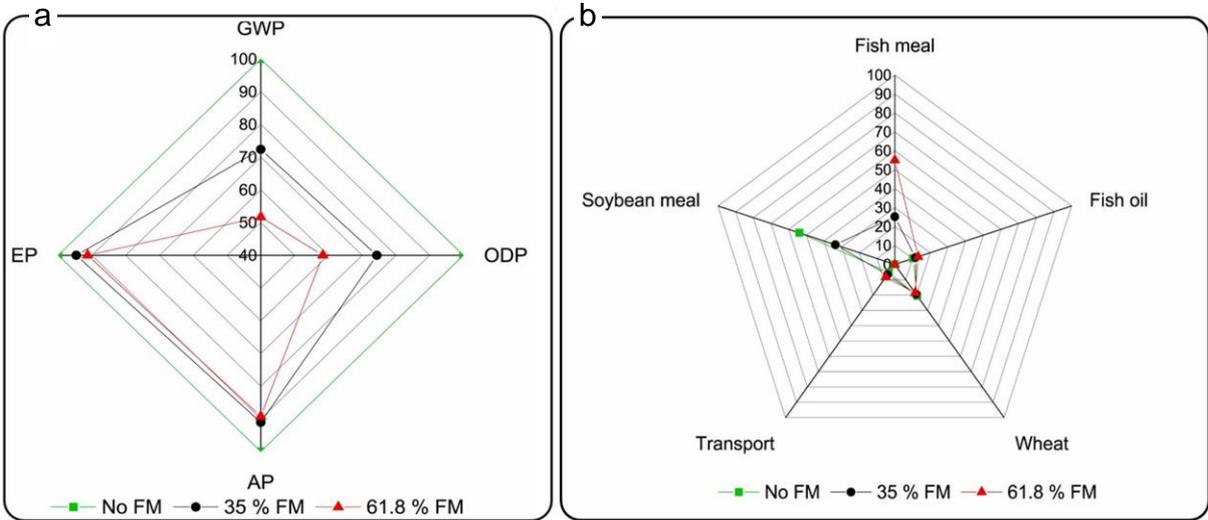
Figure 10 a) Selected categories of environmental impact associated with production of 1 kg LW rainbow trout relative to proportion of fishmeal in feed considering the FCR. GWP = global warming potential; ODP = ozone layer depletion; EP = eutrophication potential; AP = acidification potential; FM = fishmeal.
b) Percentage contribution of the main feed components and production processes to the GWP of the production of 1 kg feed. FM = fishmeal.

3.5. Discussion

The GWP of trout production under the conditions of southern Germany is similar to that reported for other flow-through farms and confirms the relatively low climate impact compared to terrestrial animal farming (Figure 11), particularly with respect to CO_2 emissions of salmonid production in open flow-through systems (Figure 12) (Aubin et al., 2009; Clune et al., 2017; d'Orbcastel et al., 2008; Samuel-Fitwi et al., 2013a). Although production is comparably efficient, there is considerable capacity for improvement through adjusting feed composition and the implementation of photovoltaic electricity generation systems. The levels of GHG emissions found in this study are lower than those previously reported for rainbow trout (Figure 12), although the No PV scenario is closer to other reported values.

The relatively low emissions may be attributed to the significantly lower FCR in the present study, as feed is the principal factor contributing to GWP. While the FCR for the model farm was 0.89, those used in the other studies were ≥1.1 (Aubin et al., 2009; d'Orbcastel et al., 2009; Samuel-Fitwi et al., 2013a).

The FCR can be affected by feed or water quality, feed management; or final fish size. The difference in the FCRs used in this research from that of (Aubin et al., 2009) can be explained by fish size, as the FCR gradually increases with fish growth (Árnason et al., 2009). (Aubin et al., 2009) analyzed trout up to 2000 g, while this study focused on
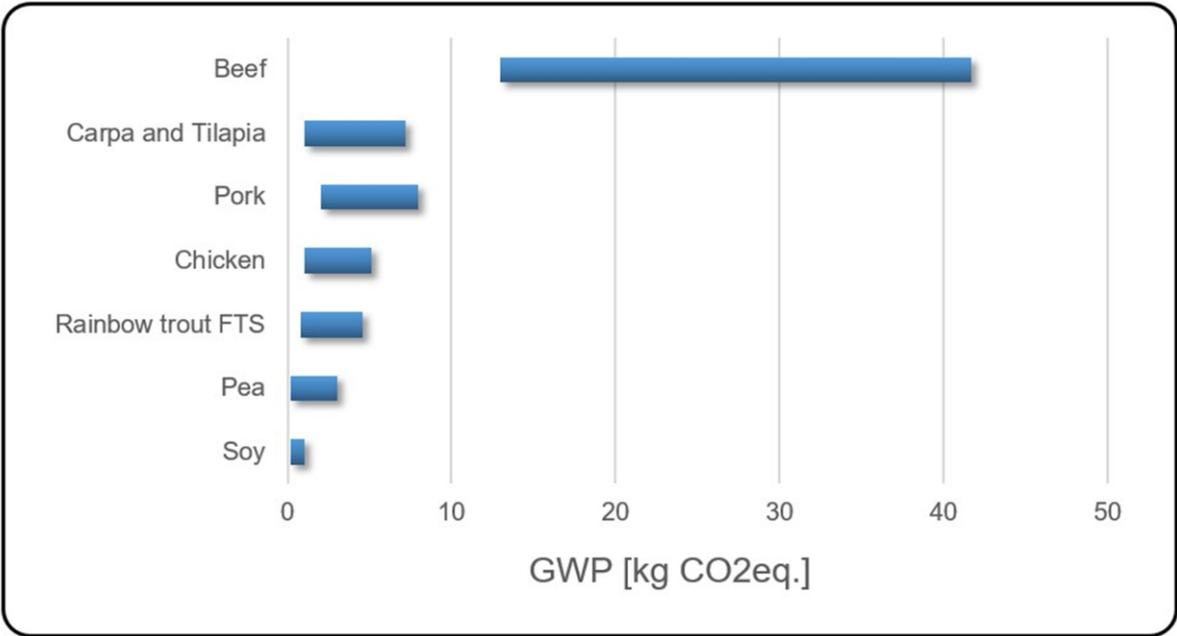
Figure 11 Global warming potential (GWP) as kg CO2eq per kg LW of rainbow trout reared in a flow-through system (FTS) and per kg product reported for animal and plant-based food products (Arrieta and González, 2019; Clune et al., 2017; Desjardins et al., 2012; McAuliffe et al., 2016; Samuel-Fitwi et al., 2013a; Wiedemann and Watson, 2018; Wiedemann et al., 2017).

plate-size trout of 380 g. (d'Orbcastel et al., 2009) found 91 % of CO_2 emissions to be associated with feed, although it is not clear whether feed, water quality or feeding management were responsible for the significantly lower feed efficiency. The higher GWP reported by (d'Orbcastel et al., 2009) could be related to the use of pumps for well water, as the electrical energy distributed in France in 2009 consisted of 50 % fossil sources while nowadays it is reduced by nuclear energy (d'Orbcastel et al., 2009; IEA, 2021). With high quality feeding management and the increased use of renewable energy in France, current values may be lower (IEA, 2021).

Samuel-Fitwi et al. (2013a) found GWP of 2.24 kg CO_{2eq} in an extensive flow-through system and nearly 3.6 kg CO_{2eq} per kg trout in intensive flow-through systems with an FCR of 0.91. This is two-fold the value in the model system of this study without photovoltaic energy generation. The energy consumption was also nearly twice that of the analyzed farm and primarily fossil based, while, at the time of the present study, the share of fossil fuel in energy sources has decreased with respect to renewable energy (Burger, 2021; Samuel-Fitwi et al., 2013a).

With the use of a portion of the production area for photovoltaic energy generation, GWP falls to levels observed for omnivorous species like tilapia reared in intensive net-cage systems (Mungkung et al., 2013; Yacout et al., 2016). Under the assumption of 100 % PV cover, the CO_2 emissions are comparable to extensive production of trout in net pens or caught pelagic fish (Grönroos et al., 2006; Sandison et al., 2021).

Environmental emissions can be reduced by regional production and direct marketing of the product (Brinker and FFS Langenargen, 2019). It is important to tailor the pro-

duction to a level of self-sufficiency in areas with suitable landscapes and water availability like that of Germany and to reduce imports of trout produced in foreign countries that inevitably increase the environmental impact related to transportation, cooling, and storage (Brinker and FFS Langenargen, 2019; Wakeland et al., 2012).

While the production of 1 kg LW trout in a flow-through system emits, on average, 2.2 kg CO_2, CO_2 emissions associated with chicken are in the same range, while pork production can also have low emissions, however depending on the farm system, they can increase up to 6.06 kg CO_{2eq}/kg LW (Clune et al., 2017; Desjardins et al., 2012; Samuel-Fitwi et al., 2013a; Wiedemann et al., 2017). Carp and tilapia GWP has a higher range, depending on the farm system (Mungkung et al., 2013; Yacout et al., 2016). Reported values for beef production can be high with reported values of nearly 29 kg CO_{2eq}/kg LW (Clune et al., 2017; Desjardins et al., 2012). A minimally processed vegetarian or vegan diet is associated with distinctly lower emissions. For example pea production emits less than 2.5 kg CO_2, while soybean production emits less than half a kilo of CO_{2eq} (Clune et al., 2017) (Figure 11).

In addition to lower CO_2 emissions compared to other meats, fish flesh has a unique composition of highly digestible proteins, EPA/DHA, vitamin D and B12, selenium, and iodine, making it a nutritionally valuable food (Béné et al., 2015; Mozaffarian and Rimm, 2006). Farmed salmonids are considered as food with high nutrient density and low environmental impact, sometimes even better than caught fish (Hallström et al., 2019). Therefore, a weekly intake of 1-2 servings of fish can already provide significant health benefits, with Omega-3 fatty acids, especially DHA, found to reduce the risk of coronary artery disease, and a high Omega-3 index being associated with longevity (Harper and Jacobson, 2005; Hu et al., 2002; McBurney et al., 2021; Mozaffarian and Rimm, 2006). Vice versa, the intake of fish oil supplemental were not found to have the same health benefit as consuming real fish (Haq et al., 2021).

Photovoltaic energy generation provides an opportunity to reduce the direct CO_2 emissions of traditional trout aquaculture toward zero. If feed production would not be considered (see discussion below), the 100 % PV scenario would have a positive carbon footprint with a CO_2 saving potential of -0.46 kg CO_{2eq}. Reduction is especially possible in typical flow-through trout farms in temperate areas as they are relatively energy efficient and use only a moderate amount of land (d'Orbcastel et al., 2009; Fornshell et al., 2012; Samuel-Fitwi et al., 2013a). Solar energy might not be applicable to extensive culture of species such as carp because of technical and financial challenges, plus

intrinsic challenges (sunlight and warming is important for natural prey production used by carps (Anton-Pardo and Adámek, 2015; C. Li et al., 2019)).

The analysis showed that the chief sources of benefit differ with impact category. With the use of photovoltaic energy, GWP is reduced mainly by limiting use of biogas, while the lower use of uranium for nuclear power and of natural gas reduces the ODP. The AP is mainly affected by the reduction in SO_x emitted in lignite flue gases and anaerobic biogas, while the EP is decreased by the lower reliance on lignite mining. Lignite makes up ~40 % of German energy sources, with some production and mining steps being harmful to the environment (Burger and Hanisch, 2022; Vlassopoulos, 2020).

Contrarily, manufacture of photovoltaic panels is energy demanding, with an amortization period of 1.6–2.1 years (Wirth, 2021). But the lifespan of 25-30 years results in a positive energy production of photovoltaic systems, which exceeds their energy required to manufacture them by many times (Wirth, 2021). Furthermore, the installation of photovoltaic systems provides multiple synergistic benefits to salmonid production. One big challenge in flow-through salmonid aquaculture is climate change-induced rise in water temperature. Shading is often not present, which then leads to temperatures rapidly exceeding optimal ranges, especially for cold-water salmonids. This causes further negative side effects like decreased solubility of O_2 (Harmon, 2009), increase in toxicity of NH_3 (Wurts, 2003), and increased virulence of pathogens such as proliferative kidney disease (Beschta, 1997; Okamura et al., 2011; Ros et al., 2022), leading to severe consequences to fish physiology and resulting in reduced growth and higher disease susceptibility. The shade provided by photovoltaic systems may reduce water temperature by 1.3°C for each 2°C air temperature reduction (Kalny et al., 2017), relevant to climate conditions in Europe (Copernicus, 2021a, 2021b). Additionally, the roofs needed for photovoltaic system installation block solar radiation, a cause of lesions and increased mortality in fish, and offer effective protection from losses and stress caused by predatory birds (Brinker and FFS Langenargen, 2019; Curtis et al., 1996; Delong et al., 1958; Littauer et al., 1997; Roberts and Bullock, 1980).

With the exception of photovoltaic systems, there are a few other methods which may be used to improve electricity use on aquacultural farms, like the building of small hydroelectric power stations on the production sites which utilize the natural gradients of the water flow as energy source. Another option could be the use of excess photovoltaic energy to produce oxygen on site using an oxygen generator to reduce the dependence on external liquid oxygen. The EU promotes sustainability of aquaculture by

53

providing funds like EMFAF (Directorate-General for Maritime Affairs and Fisheries, 2024).

The results showed a high proportion of the assessed environmental impact of feed to be associated with soy, wheat, and fish meal. Overfishing and sea floor degradation (Allan et al., 2005; Auster et al., 1996; Coll et al., 2008; Jackson et al., 2001; National Research Council, 2002) were not considered but estimated to be small, since fish for fish meal production is caught almost exclusively in pelagic regions, targeting small species such as anchovy, menhaden, and sardines and primarily using purse seines or dip nets (Chagaris et al., 2020; Kolding et al., 2019; Tacon and Metian, 2008). Furthermore, those fish stocks are generally well managed and catches certified (Arlinghaus et al., 2021). The 61.8 % fish meal diet formulation was found to have the lowest effects on the assessed categories. The use of unprocessed (waste) parts of edible fish or bycatch for fish meal production is increasing (Funge-Smith et al., 2005; Sotolu, 2009; Ytrestøyl et al., 2015). Wild fish currently account for ~75 % of the raw material in fish meal and oil (I.F.F.O., 2021). The fish in:fish out ratio of some important farmed species has been dramatically reduced, in salmonids from 3.8 (1995) to 1 (2020) (I.F.F.O., 2021; Kok et al., 2020), meaning that for every kg of fish fed, 1.0 kg of fish is produced.

There is finite limit to fish captured from the wild. According to the FAO, 52 % of marine fish stocks are fully exploited and 17 % overexploited (FAO, 2011), driven by catches for aquaculture and by increasing the demand for fish for human consumption. Research should not only concentrate on the optimization of fish meal and -oil production (Ghamkhar and Hicks, 2020), but also on alternatives like single cell oils and proteins or insect-based foods (Cressey, 2009; Goldburg and Naylor, 2005).

The use of some plants as fish meal replacement can lead to high GHG emissions, mainly related to cultivation and land use. The current demand for soy, for instance, exceeds its production, bringing transformation of land and deforestation of rainforests (Dros, 2004; Graesser et al., 2018; Song et al., 2021). Commercial cultivation of plants involves use of pesticides, which can leach into soil and groundwater, affecting biodiversity and the environment over large scales (Glick and Bashan, 1997; Pimentel et al., 1992). A large proportion of global wheat and soy is transported long distances (Dros, 2004; FAO, 2019; Sharma et al., 2015). Soy, as other plants, needs to be heavily processed to be an adequate replacement for fish meal (Francis et al., 2001). For example, the lectins in soybeans were found to harm intestines of salmonids (Dersjant-

Li, 2002; Hendriks et al., 1990). Highly processed plant-based foods for human consumption can exhibit a larger environmental impact than animal products, especially relative to their nutrient content (Chai et al., 2019; González et al., 2011; Smedman et al., 2010).

Further options to expand the sustainability of fish feed could include the use of technologies such as genetic manipulation of plants to minimize their anti-nutritive content, reduce pesticide reliance, and increase resistance to extreme weather (Grover et al., 1998; Gupta et al., 2015; Kwon et al., 2001; Muzquiz et al., 2012; Šamajová et al., 2013).

Ghamkhar and Hicks (2020) have also shown, that diets lacking fish meal and oil do not necessarily have a reduced environmental impact and substituents need to be considered carefully. One suggested solution in recent literature is the use of insect meal (English et al., 2021; Maiolo et al., 2020; Sánchez-Muros et al., 2014; Stenberg et al., 2019). However, studies have shown that the positive effects of reduced agriculture and fishing are currently outweighed by the energy and feed necessary to produce adequate quantities of insects (Le Féon et al., 2019; Maiolo et al., 2020; Sánchez-Muros et al., 2014; Stenberg et al., 2019; St-Hilaire et al., 2007b).

As a consequence, insects have a higher environmental impact than soy and fish meal regarding protein content per kg meal (Thévenot et al., 2018). Smetana et al. (2016) suggest the use of feed by-products to avoid higher impacts but further research is needed to investigate the environmental impact of sustainably produced insect-based fish feed.

3.6. Conclusion

Fish can be a highly nutritional meat product with comparably low emissions, although there is room for improvement. The use of photovoltaic energy generation can reduce the environmental impact of a fish farm by limiting the use of fossil energy sources and producing excess renewable energy. The formulation of a sustainable fish feed is a goal, with this research showing that plant-based alternatives are not currently associated with a better GWP profile than fish meal. Future research should concentrate on the sustainable use of fish meal accompanied by alternatives such as plants with low anti-nutritive properties and low processing costs and possibly new alternatives like sustainably produced insects.

3.7 Supplements

Table TS7 Feed composition as given in Brinker and Reiter (2011).

	No FM	35% FM	61.8% FM
Crude protein	476	437	421
Crude lipid	172	187	201
Crude starch	16.90	14.60	15.50
Total phosphorous	0.99	1.08	1.52
Gross energy	23.90	23.60	23.70
Digestible energy	17.20	17.30	18.60
Diet ingredients			
SC fishmeal[a]		175	309
SA fishmeal[b]		175	309
Soycomil[c]	351		
HP soya[d]		175	
Wheat gluten[e]	253	142	
Corn gluten[f]	25		
Wheat[g]	100	100	100
Wheat starch[h]	61	73	136
Fish oil[i]	171.50	155.55	141.55
Mineral premix[j]	1.10	1.10	1.10
Vitamin premix[j]	1.10	1.10	1.10
L-Lysine HCL[k]	7.50		
Vitamin C 35%[l]	0.43	0.43	0.43
Vitamin E 50%[l]	0.40	0.40	0.40
Dl-methionine[m]	1.55		
MCP[n]	24.50		
Betafin[o]	0.42	0.42	0.42
Inositol[j]	0.50		
Yttrium premix[j]	1	1	1

[a] SR fishmeal, SR-mjöl, Iceland (mainly blue whiting, low temperature drying); Soybean protein concentrate with a crude protein content of about 62%.
[b] Super Prime fishmeal, Peru (mainly anchovy).
[c] Archer Daniels Midland Company (ADM), USA.
[d] Oelmühlen Hamburg Aktiengesellschaft; Soybean meal (dehulled) with about 48% of crude protein.
[e] Amytex 100 (80% protein, 5% fat, max 1.5% ash), Amylum, Belgium.
[f] Corn gluten meal (60% protein), Tate&Lyle, USA.
[g] Bread-making quality, (Triticum aestivum, origin: Sweden).
[h] Cerestar Deutschland GmbH, Germany.
[i] SR fish oil, SR-mjöl, Iceland (mainly blue whiting).
[j] Farmix, Putten, The Netherlands.
[k] Ajinomoto Eurolysine, France.
[l] BASF, Ludwigshafen, Germany.
[m] Degussa, Germany
[n] Kermira GrowHow Oyi, Finnland.
[o] Betafin S1, Danisco, Denmark; 96% betaine anhydrate

Table TS8 Feed composition used in the LCA.

Feed ingredient Origin	Unit	No FM	35% FM	61.8% FM
Fish meal NO	kg		0.175	0.309
Fishmeal PE	kg		0.175	0.309
Fish oil NO	kg	0-1715	0.15555	0.14155
Wheat grain US	kg		0.100	0.100
Wheat starch NL	kg	0.061	0.073	0.136
Vitamin animal feed FR	kg	0.02945	0.0045	0.309
Soybean meal RoW	kg	0.351	0.175	
Wheat gluten meal BE	kg	0.253	0.142	
Maize gluten meal US	kg	0.025		
L-Lysine HCl animal feed FR	kg	0.0075		
DL-Methionine animal feed RER	kg	0.00155		
Electricity, medium voltage DK	kWh	0.000011	0.000011	0.000011
Heat	kWh	0.148	0.148	0.148
Transport freight lorry >32 metric ton, euro5	tkm	1048	1048	1048

Table TS9 Photovoltaic scenarios used in the LCA (PV = Photovoltaic).

Process	No PV	40% PV	100 % PV
On-Farm vehicles (p)	0.000016	0.000016	0.000016
Oxygen (kg)	0.67	0.67	0.67
35% FM Feed (kg)	0.89	0.89	0.89
Diesel (L)	0.007281	0.007281	0.007281
Water (m³)	30	30	30
Land use (m²a)	0.09	0.09	0.09
Electricity input (kWh)	0.16	0.16	0.16
Energy output (kWh)	-	0.938294	1.64558175

Table TS10 Percentage contribution of the different components to the emissions of the production of 1 kg feed (FM = Fishmeal, AP = Acidification potential, EP = Eutrophication potential, ODP = Ozone layer depletion, GWP = Global warming potential).

Impact categories	AP (kg SO$_{2eq.}$)			EP (kg PO$_{4eq.}$)			ODP (kg CFC$_{11eq.}$)			GWP (kg CO$_{2eq.}$)		
FM	No	35%	61.8%	No	35%	61.8%	No	35%	61.8%	No	35%	61.8%
Soy	16	8		27	18		21	15		54	34	
Fish meal		33	54		10	23		13	32		25	55
Fish oil	16	15	13	4	5	6	3	3	4	10	12	13
Wheat	45	36	26	58	64	68	38	36	18	20	19	19
Transport	6	6	6	2	2	3	22	32	44	5	7	8
Heat	0	0	0	0	0	0	0	0	0	2	3	3
Other additives	10	1	1	5	1	1	16	2	2	7	1	1
Maize	6			4			0			1		
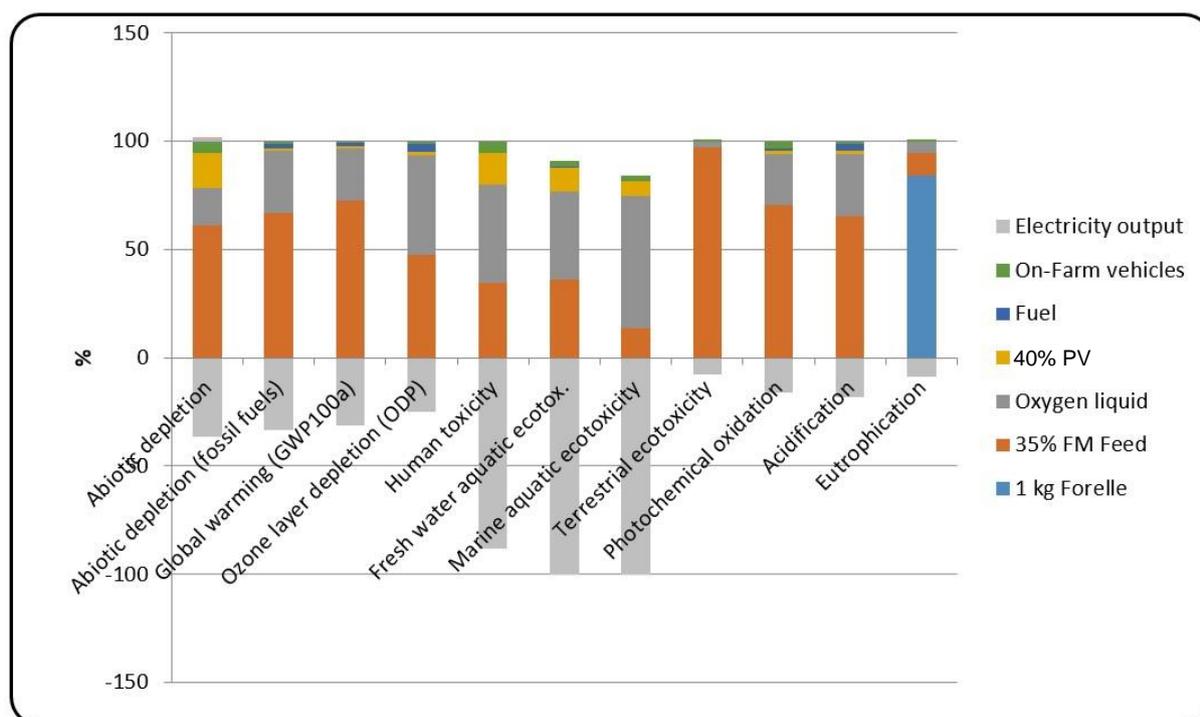
Figure S5 Whole results for the model farm using 40% photovoltaic system and 35% fishmeal feed.

4. Performance and physiological consequences of totally replacing soy protein concentrate in rainbow trout (*Oncorhynchus mykiss*) diets with black soldier fly (*Hermetia illucens*) larval meal

4.1. Abstract

Recent years have seen increasing moves to substitute the fish content of aquafeeds with plant based ingredients. However, challenges inherent in the use of such ingredients drive an ongoing search for sustainable and cost effective alternatives. To study the potential of high insect protein content in rainbow trout diets, a dose response study was set up to assess the effects of replacing up to 100 % of soy protein concentrate (SPC) with a meal of black soldier fly (*Hermetia illucens*, BSF) larvae. In a 10 week feeding trial, a homogeneous group of 1100 rainbow trout (initial body weight: 135.8 ± 15.3 g) were supplied with 10 different diets: a commercial soy based control and variants in which the SPC content was replaced with increasing proportions of BSF larval meal. All diets also incorporated the same low fishmeal content (7.5 %) and were tested with and without a faecal binder treatment. At the end of the experiment, growth performance, feed utilization, organosomatic indices, and fillet yields were determined alongside histological and transcriptomic analysis of the liver and intestine. Results indicated that substitution with BSF larval meal (BSFLM) was associated with increased feed intake and a significant, although non-linear, improvement in growth, hinting at nutritional deficiencies in the commercial SPC controls. However, fish fed with insect free diets exhibited greater protein retention and feed conversion ratio (FCR). No significant differences were apparent in intestinal or liver histology, gene expression or fillet processing yield between treatments, indicating that even a complete replacement of SPC with BSFLM can take place without compromising rainbow trout welfare or productivity.

4.2. Introduction

Feeding the world's growing population without exceeding the planet's environmental limits is one of the greatest challenges of the 21st century. Aquaculture plays a crucial role in meeting global demand for food, contributing to more than 50 % of globally consumed fish (FAO, 2024g). However, as the sector continues to expand and become

more integrated into the global food system, concerns about its environmental impact will increase accordingly (Newton et al., 2023). Sourcing high quality, cost efficient feed ingredients in an intensifying sector, while meeting sustainability standards has become a significant challenge. Over the years, efforts have been made to maintain nutritional efficiency while reducing the weight of dependency on marine ingredients for aquafeeds, particularly for high valued carnivorous species (Newton et al., 2023). Several vegetable protein alternatives are available to replace fishmeal (FM) in commercial aquaculture feeds, with soy based variants being best established (van Riel et al., 2023). In 2020, approximately 40.5 % of salmonid diets were constituted of plant-based protein sources, with soy protein concentrates (SPCs) constituting approximately 21% of feeds formulations (Aas et al., 2022a, 2022b). The production of SPCs comes at a relatively high cost, due to the processing required to maximize digestibility and reduce levels of anti-nutrients (Li et al., 2015). SPCs are used to mitigate the nutritional disadvantages of soybean meal (SBM), such as poor palatability, insufficiencies of essential amino acids (EAAs), minerals and vitamins (Zhu et al., 2020) and high levels of anti-nutritional factors (ANFs) linked to inflammatory responses in the distal intestine of fish (Merrifield et al., 2011). Although SPCs can successfully substitute FM in aquafeeds, as earlier suggested (Brinker and Reiter, 2011; Drew et al., 2007; Olli and Krogdahi, 1994), soy production is associated with numerous factors that render it a less desirable choice for aquaculture feeds. Its use impacts feed food competition directly, since it could be used as a human food source and indirectly, by increasing the area of agricultural land used for feed production rather than human food production (van Riel et al., 2023). Soy production is also associated with environmentally problematic practices such as deforestation and the use of fertilizers and pesticides (Newton and Little, 2017; Pelletier et al., 2018). Nowadays, aquafeeds are the biggest contributor to the environmental impacts of aquaculture (Wind et al., 2022), amounting to approximately 57 % of the sector's total emissions, mostly through crop production and marine resource extraction for ingredients (MacLeod et al., 2020).

Incorporating insects into animal nutrition is not a novel concept (DeFoliart, 1989), but it has received considerable renewed attention in recent years. Scientific evaluations of their potential in alternative aquafeeds has significantly increased, pointing to a significant contribution in the future of the aquafeed industry (Hua et al., 2019; Tran et al., 2022; van Huis, 2020). As part of a natural diet, insects provide a sound balance of

amino acids, lipids, vitamins, and minerals for most farmed salmonids (Nogales- Mé-rida et al., 2018), such as rainbow trout (*Oncorhynchus mykiss*). In Europe, products from seven different insect species are currently approved for use in aquaculture diets, under an amendment to Commission Regulation (EU) 2017/893. Of these, the best studied are black soldier fly (*Hermetia illucens*, BSF), yellow mealworm (*Tenebrio molitor*) and common housefly (*Musca domestica*) (Gasco et al., 2021). Recent reviews identify insect meal as one of the most promising and sustainable alternatives for fish feeds in the forthcoming years (Hua, 2021; Hua et al., 2019; van Huis, 2020).

In the context of animal feed BSF larval meal (BSFLM) is credited with several advantages over other insect ingredients (Gasco et al., 2021; Tran et al., 2022). Besides being a polyphagous and a particularly protein rich organism, the intestinal tract of BSF contains considerable amounts of amylase, lipase, and protease activity, rendering it a high quality bioconverter (Barragán-Fonseca et al., 2017). BSF larvae are fast growing, able to perform rapid conversion of feed to body mass (van Huis, 2013). For salmonids, a BSFLM diet offers a suitable, well balanced amino acid profile including EAAs, and a potentially valuable lipid fraction, rich in both EPA and DHA (English et al., 2021). In addition, a plastic development trajectory means BSF can be easily manipulated by diet (Oonincx and Finke, 2021), to express an optimal nutrient profile in its larval form. BSF larvae can contain between 40-45 % protein and 26-35 % lipids as a proportion of a dry weight, making it a specially promising alternative for carnivorous fish, which typically require 45-55 % crude protein and 16-30 % crude lipid in their diets (Barragán-Fonseca et al., 2017; Jobling, 2012; Nogales- Mérida et al., 2018). BSF larvae are also rich in bioactive compounds, including some with antibiotic properties, reducing the presence of bacterial pathogens and enhancing gut health (Xia et al., 2021). Furthermore, BSF can be grown in organic waste streams using less land, and causing relatively lower of greenhouse gas emissions, though this method is not currently permitted in the EU (Makkar et al., 2014; Oonincx et al., 2015; van Huis, 2013).

The applicability of BSFLM as a feed ingredient in the salmonid industry has already shown promising, if controversial, results (Weththasinghe et al., 2021a). The majority of published studies focus on partial replacement of FM with BSFLM (Belghit et al., 2018; Couto et al., 2022; Melenchón et al., 2022; Stadtlander et al., 2017), while fewer publications reported its potential for substituting plant derived ingredients (Dietz and Liebert, 2018; Hossain et al., 2021; Randazzo et al., 2021a). Furthermore, although considerable debate continues regarding optimal replacement levels, the potential of

BSFLM as a complete substitute for SPC in salmonid diets has yet to be investigated (Weththasinghe et al., 2021a).

To evaluate the effects of substituting up to 100 % SPC with BSFLM, a 10 week dose-response experiment was conducted. For that purpose, 10 balanced experimental diets were formulated in which SPC was replaced with increasing levels (0 %, 25 %, 50 %, 75 %, and 100 %) of BSFLM, with and without the addition of 0.3 % guar gum (GG) as a faecal stability binder. The present study spotlights the effects of these modifications by assessing growth performance, feed utilization and health of rainbow trout. Histological and gene expression analysis were carried out to investigate possible physiological consequences of using BSFLM in fish diets. An account of the consequences of dietary treatments on faecal rheology will form part of a follow up publication.

4.3. Material and methods

Experimental diets. This study deployed 10 iso-lipidic (29 % crude lipid) and iso-nitrogenous (46.7 % crude protein) extruded diets (pellet size 4.5 mm) whose ingredients and proximate composition are shown in Table 9. The diets were formulated to meet the specific nutritional requirements of rainbow trout (Jobling, 2012) and to test the effects of SPC substitution with increasing levels of BSFLM on fish performance and health. A soy based commercial standard diet (control) was used as a reference, while treatments incorporated four increasing substitution levels of BSFLM at 25 (25BSF), 50 (50BSF), 75 (75BSF), and 100 % (100BSF). An additional 0.3 % GG was added to five of the resulting duplicate diets (control+, 25BSF+, 50BSF+, 75BSF+, and 100BSF+). To account for nutritional deficiencies, all diets were balanced with respect to EAAs and supplemented with selected additives, such as vitamin and mineral premixes. Fish oil (FO) and rapeseed oil were added to provide sufficient long-chain unsaturated fatty acids (LC-PUFAs) and the diets also included 0.2 % indigestible yttrium oxide (Y_2O_3) as an inert marker. All the manufacturing details are property of Skretting (Stavanger, Norway), and the proximate dietary compositions are provided as estimated values for near infrared spectroscopy (NIRS). All feeds were kept in an aerated cooling chamber at a constant temperature of 5 °C to preserve their properties.

Feeding trial. The feeding trial was conducted at the Fisheries Research Station of Baden-Württemberg (Langenargen, Germany) over a 10 week period, following the

recommendations of the German and European guidelines on the protection and welfare of animals used for scientific purposes. During an acclimatization of 3 weeks, a homogenous group of 1100 rainbow trout from a local strain (initial body weight (IBW): 135.8 ± 15.3 g), was fed with a commercial feed (EFICO Enviro 920 Advance, BioMar Group, Denmark). After acclimatization, the fish were randomly distributed into 2 semi-technical systems comprising a total of 20 green circular fiberglass tanks (10 each) with a volume of 0.33 m³. The 2 systems were operated in flow-through mode, using sand filtered freshwater from Lake Constance (with a constant flow rate of 5 L/min). During the trial, the photoperiod was fixed at 12L:12D, *i.e.*, 12 hours light and 12 hours darkness (Lumilux® daylight lamps provided approximately 160 lux at the water surface between 07:30 a.m. and 07:30 p.m.), with a 30 min sigmoidal transition period designed to simulate dawn and dusk. In order to maintain stable water parameters, the system was equipped with probes that continuously monitored temperature (Temperature Probes, Oxyguard, Denmark) and dissolved oxygen (Oxygen Probes, OxyGuard, Denmark), which were maintained automatically between 9-11 °C and 11-11.5 mg/L respectively. The fish were hand fed twice a day (8:30 a.m. and 3:30 p.m.), 6 days per week (Monday to Saturday), until apparent satiation. Thus excess feeding was kept to a minimum, resulting in a daily feed intake of 1.1 % of the body weight. All treatments were tested in duplicate, and the feed allocated to each tank was quantified daily. The animals were all female rainbow trout, to eliminate the possibility of precocious maturation and minimize variability by sex related effects.

Sampling. At the beginning and end of the experiment, all fish were individually weighed (g) and examined for external and internal macroscopic abnormalities. A pool of 20 fish from the initial stock and a pool of 5 fish from each tank at the end of the trial were randomly sampled for transcriptome analysis of the proximal intestine. The samples were placed in cryotubes and stored at 80 °C until required for analysis. During the final sampling, 2 additional fish per tank were randomly sampled for histological analysis and tissues were collected from the liver and the proximal and distal intestine. Faecal material from all tanks was collected by dissection of distal intestine to perform the digestibility measurements. The faeces were immediately frozen at 20 °C until analysis. In addition, 10 fish per tank were sampled and filleted by an experienced person to assess processing yields.

Table 9 Formulation and proximate composition of the experimental diets.

Diets / Ingredients %	Control	25 BSF	50 BSF	75 BSF	100 BSF	Control+	25 BSF+	50 BSF+	75 BSF+	100 BSF+
Soy protein concentrate	30.00	20.00	10.14	5.00	-	30.00	20.00	9.10	5.60	-
Black soldier fly meal[1]	-	10.00	20.00	30.00	40.00	-	10.00	20.00	30.00	40.00
Fish meal	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Soybean meal	-	-	5.00	5.00	-	-	-	5.00	5.00	-
Wheat	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Wheat gluten	16.58	18.45	17.35	17.71	18.71	15.00	18.00	18.12	16.9	18.3
Guar meal	5.00	5.00	5.00	1.48	-	6.91	5.65	5.00	0.72	-
Fish oil	9.30	9.73	10.53	11.55	16.40	9.18	9.67	10.50	12.87	16.24
Rapeseed oil	14.23	12.25	10.10	8.22	3.00	14.04	12.27	10.11	7.00	3.00
Guar gum	-	-	-	-	-	0.30	0.30	0.30	0.30	0.30
Sum additives and premixes	11.39	11.06	8.38	7.54	8.38	11.07	10.61	8.36	8.11	8.67
Proximate composition (% DM)										
Dry matter (DM), %	92.70	92.90	93.20	92.40	93.40	93.60	93.00	93.30	93.20	92.70
Crude protein	46.17	46.50	46.78	46.86	47.00	46.26	46.24	46.95	46.67	47.14
Crude fat	28.91	28.85	28.86	29.00	29.76	28.63	28.71	28.51	28.54	29.77
Ash	4.85	4.84	5.36	5.95	6.96	4.91	4.84	5.36	6.22	6.80

Diets proximal composition was provided as estimated values for near-infrared spectroscopy (NIRS). [1]Black soldier fly (*H. illucens*, BSF) meal: 15% crude fat; 56% crude protein; chitin content not estimated.

All fish were sampled by first stunning with a sharp blow to the head followed by a sacrificial gill cut, following the recommendations of the German and European guidelines on the protection and welfare of animals used for scientific purposes (TierSch-VersV, 1 August 2013 and Directive 2010/63/EU of the European Parliament of the European Union Council).

Histological Analysis. Histological evaluation of tissues from the liver, proximal, and distal intestines was performed at the University of Veterinary Medicine in Hannover, using standard methods (Miebach et al., 2023). After dissection, tissue samples were fixed using 4 % buffered formaldehyde (pH 7.2). Before analysis, the tissues were dehydrated in series of graded ethanols, and embedded in paraffin to form solid blocks. Posteriorly, histological cross-sections of 2 µm were cut, mounted, deparaffinised in butyl acetate and isopropanol and stained with haematoxylin eosin (HE) or alcian blue PAS (AB-PAS). The stained sections were used to measure the thickness of *stratum granulosum*, and *stratum compactum* (4 measurements/slide). Inflammatory cell infil-

tration into *lamina propria* was graded using a predetermined scoring system, (0: absent; 1: low; 2: moderate; and 3: high). AB-PAS stained sections were used to determine the density, as well as the goblet cells filling levels, also based on a score from 1 to 3, representing low to high respectively. The entire analysis was randomized, and blindly evaluated by light microscopy.

Microarray hybridization and quantitative PCR analysis. Individual RNA samples isolated from the proximal intestine of fish provided with 0 and 100 % BSF with or without additional 0.3 % GG (control, control+, 100BSF, and 100BSF+) were converted to Cy3-labeled cRNA and hybridized with 8×60 K Agilent Salmon Oligo Microarrays (ID 020938, Agilent Technologies; GEO platform: GPL21057) following the Agilent 60-mer oligo microarray processing protocol of Martorell-Ribera et al. (2022). A G2505C Microarray Scanner System (Agilent Technologies) was used to scan the fluorescence signals of the hybridized Agilent microarrays at a resolution of 2 µm. The full complement of microarray data was deposited in the NCBI database Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/; accession: GSE243211). The reliability of the microarray-predicted expression difference was validated using reverse transcription-quantitative PCR (RT-qPCR). A dlgap4-specific oligonucleotide primer pair (exon-exon junction spanning sense primer: 5' GGAGAACGGCAGGCCAC-TCA 3'; antisense primer: 5' GAGTACGCAGTGGTGCGGCA 3') was designed using the Pyrosequencing Assay Design software (v.1.0.6; Biotage, Uppsala, Sweden) to amplify a 186-bp amplicon. Rps5 (ribosomal protein S5), rna18s (18S ribosomal RNA (Köbis et al., 2017)), actb (beta actin (Rebl et al., 2009)), and eef1a1 (eukaryotic translation elongation factor 1, alpha 1 (Bowers et al., 2008)) were chosen as reference genes (with coefficients of variation, CV < 0.20). Individual RNA samples from rainbow trout (system A and system B, each n = 4) fed with 100BSF, 100BSF+, control, and control+ were reverse-transcribed into cDNA using the SensiFAST cDNA Synthesis Kit (Bioline/Meridian Bioscience). The qPCR analysis was conducted with the Light-Cycler-96 system (Roche, Mannheim, Germany) using the SensiFAST SYBR No-ROX Kit (Bioline/Meridian Bioscience, Luckenwalde, Germany), according to the following program: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s; primer annealing at 60 °C for 15 s; elongation at 72 °C for 15 s; and fluorescence measurement at 72 °C for 10 s. Melting curve analysis validated the amplification of the target fragment. In addition, amplicons were visualized on 3 % agarose gels to evaluate product size and quality.

Apparent dry matter digestibility. To determine dry matter (DM) and yttrium oxide (Y_2O_3) contents, faecal matter dissected from the distal intestines was first lyophilized and homogenized. The DM content of each sample was obtained from the ratio of dry to wet weight, before and after lyophilisation. After sample preparation, the Y_2O_3 levels were determined using inductively coupled plasma mass spectrometry (ICP-MS) at the federal chemical analysis service of Baden-Württemberg (Sigmaringen, Germany). The apparent digestibility coefficient (ADC) of dry matter was calculated as follows:

$$ADC\ (\%, DM) = 100 - \frac{100 * Y_2O_{2(diet)}}{Y_2O_{2(diet\ faeces)}}$$

Analytical determinations. The collected data was used to determine the specific growth rate (SGR), thermal-unit growth coefficient (TGC), feed conversion ratio (FCR), protein efficiency ratio (PER), total feed intake (TFI), hepatosomatic index (HSI), viscerosomatic index (VSI), head on gutted yield (HOG) and skin on fillet yield (SK ON FIL) obtained as follows:

- $SGR\ (\%\ per\ day) = \frac{(\ln FBW(g) - \ln IBW(g))}{time(days)} * 100$

- $TGR = ((FBW^{\frac{1}{3}}(g) - IBW^{\frac{1}{3}}(g)) * \sum T(°C) * time(days)$

- $TFI\ (g) = \sum daily\ consumed\ feed\ (g)$

- $FCR = \frac{total\ feed\ intake\ (g)}{weight\ gain\ (g)}$

- $PER\ (\%) = \frac{weight\ gain(g)}{protein\ intake\ (g)} * 100$

- $HSI\ (\%) = \frac{liver\ weight\ (g)}{body\ weight\ (g)} * 100$

- $VSI\ (\%) = \frac{Viscera\ weight\ (g)}{body\ weight\ (g)} * 100$

- $HOG(\%) = \frac{head - on\ gutted\ weight\ (g)}{body\ weight\ (g)} * 100$

- $SK - ON\ FIL\ (\%) = \frac{fillet\ with\ skin\ weight(g)}{body\ weight\ (g)} * 100$

Data analysis. Growth performance, feed utilization, organosomatic indices, histological analysis, and processing yields data were expressed as grand marginal means ± SD, considering each tank as an experimental unit. All data was tested for homogeneity and normality. Differences between treatments were detected by a generalized linear mixed model (GLMM), considering insect protein percentage and GG as independent variables, with tank as a random effect factor. When the results showed significance, the means between treatments were compared using a contrast

post-hoc test. The analysis described above was performed using JMP® Pro, Version 17.2.0 (2023) SAS Institute Inc., Cary, NC, 1989-2024. Microarray image files were read and background corrected using the Agilent Feature Extraction Software (FES) 10.7.3.1. Features that passed this quality control were further analyzed with the limma package (Smyth GK. "limma: Linear Models for Microarray Data" (Gentleman et al., 2005), in RStudio (2022.02.3). Following quantile normalization, pairwise comparisons of the transcript abundances from the individual datasets from 100BSF/100BSF+ vs. control/control+ were employed. Hierarchical/K-means clustering and heatmap matrix analysis were performed using the R packages pheatmap, factoextra, and cluster. To control for false discovery rate, p-values were adjusted (Benjamini and Hochberg, 1995) returning only one feature (ID: A_05_P448532) with an adjusted p-value (q-value) of < 0.05. This feature was annotated using the Basic Local Alignment Search Tool (BLAST) (tax id: 8022; coverage and sequence identity: > 98 % of > 90 %, E value: 1×10^{22}). The RT-qPCR data were extracted using the LightCycler-96 analysis software v. 1.1.0.1320 (Roche) and normalized against the geometric mean of individual reference gene expression values. The GraphPad Prism software (v10.0.2) was used for the statistical analysis (Student's t test) of normalized RT-qPCR data. The results were considered significantly different at $p < 0.05$ level.

4.4. Results

Fish performance and feed utilization. All experimental diets were well accepted by rainbow trout and no differences in behavior were observed during hand feeding. Regardless of treatment, fish performed well in terms of growth and feed utilization for all parameters measured, as shown in Table 10. At the end of the experiment, fish growth—expressed as FBW, WG, SGR, and TGC—exhibited no significant differences between treatments, except for SGR, which presented significantly lower values (between 1.34 ± 0.05 and 1.35 ± 0.02 %/day) for groups fed control diets ($F_{(4,10)} = 4.97$; $p = 0.018$), compared to the test groups (between 1.42 ± 0.06 and 1.48 ± 0.03 %/day). The control groups also exhibited significantly lower TFI values ($F_{(4,10)} = 4.97$; $p = 0.018$), compared to fish fed with increasing levels of BSFLM. In terms of feed utilization, even though they were consumed less, the control treatments showed a better FCR, between 0.75 ± 0.01 and 0.76 ± 0.02 ($F_{(4,10)} = 6.79$; $p = 0.007$), and higher PER, between 3.07 ± 0.06 and 3.08 ± 0.05, differing significantly from the remaining groups ($F_{(4,10)} = 11.53$; $p = 0.001$). Increasing proportions of dietary BSF did

appear to impact on the ADCDM, with values between 72.0 ± 0.34 and 77.4 ± 0.48 % ($F_{(4,10)}$ = 3.12; p = 0,066). The addition of 0.3 % GG did not appear to exert any significant effect on any of the analyzed parameters. All reported mortality was attributable to occasional escapes from the tanks, with survival rates varying between 99.09 and 100 %, with no significant differences between treatments.

Organosomatic indexes and processing yields. At macroscopic level, all trout appeared to be in good health with no significant lesions or deformities observed during visual inspections of external body condition. Livers and intestines appeared normal in shape and color, and no signs of apparent necrosis were observed. HSI (between 1.45 ± 0.01 and 1.87 ± 0.09 %) and VSI (between 14.45 ± 2.05 and 16.84 ± 0.77 %) did not differ within treatments. The determination of HOG carcass and SK-ON FIL yields showed values between 83.16 ± 0.77 and 85.55 ± 2.05 % for HOG and between 49.99 ± 0.75 and 51.70 ± 0.89 % for SK-ON FIL, and were not influenced by the different diets (Table 11).

Histological Investigations. Histological examinations of the intestinal tract were performed to check for signs of hepatic inflammation and lipid accumulation by the presence of hepatocyte steatosis, as well as other disorders and inflammatory responses to the experimental diets (Table 12, Figure 13 and Figure 14). No influence of increasing dietary levels of BSF was observed on the structural parenchyma of liver tissues. However, liver sections from all treatments presented a low to moderate degree of lipid accumulation, as evident from the levels of hepatocyte vacuolization with a mean score from 0.6 to 1.5 (Figure 13A). No significant inflammatory responses were observed on liver tissue, with mean score values from 0.1 to 1 for cell infiltration (Figure 13B). No significant effect of increasing dietary levels of BSF was observed on the degree of vacuolization ($F_{(1,17)}$ = 2.682e-6; p = 0.999) or inflammation ($F_{(1,20)}$ = 0.31; p = 0.582) between experimental treatments.

The intestinal structure was found to be normal for farmed rainbow trout, with no apparent histopathological alterations of the proximal or distal tissues. Proximal and distal cross sections revealed mean diameter values of 3176 – 4399 µm and 5052 – 6587 µm respectively, with no statistical significance between treatments ($F_{(4,9)}$ = 0.50;

Table 10 Growth performance, feed utilization and apparent dry matter digestibility of rainbow trout fed with experimental diets. Values are presented as means ± standard deviation (n=2). P: Percentage of Insect Protein (0 (control), 25, 50, 75, 100); GG: Guar Gum (additional GG); P*GG: Interaction of P with GG. IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, SGR: Specific growth rate, TGC: Thermal-unit growth coefficient, TFI: Total feed intake, FCR: Feed conversion ratio, PER: Protein efficiency ratio, ADCDM: Apparent digestibility coefficient of the dry.

Diets	IBW (g)	FBW (g)	Survival (%)	WG (g)	SGR (%/day)	TGC	TFI (g)	FCR	PER (%)	ADC$_{DM}$ (%)
Control	138.35 ± 16.09	339.00 ± 55.44	100.00 ± 0.00	11035.90 ± 691.27	1.34 ± 0.05[a]	2.72 ± 0.27	8390.80 ± 360.06[a]	0.76 ± 0.02[a]	3.07 ± 0.06[a]	73.66 ± 2.80
Control+	135.46 ± 13.71	327.32 ± 54.24	100.00 ± 0.00	10551.80 ± 259.93	1.35 ± 0.02[a]	2.72 ± 0.41	7907.20 ± 324.56[a]	0.75 ± 0.01[a]	3.08 ± 0.05[a]	71.95 ± 0.34
25BSF	134.44 ± 15.25	346.99 ± 48.50	100.00 ± 0.00	11690.55 ± 135.69	1.43 ± 0.06[b]	2.91 ± 0.49	8969.95 ± 105.57[b]	0.77 ± 0.00[b]	3.02 ± 0.00[b]	75.34 ± 1.12
25BSF+	136.93 ± 15.41	351.95 ± 52.32	99.09 ± 1.29	11712.24 ± 724.59	1.44 ± 0.06[b]	2.94 ± 0.26	9187.45 ± 424.33[b]	0.78 ± 0.01[b]	2.96 ± 0.05[b]	74.40 ± 2.01
50BSF	134.84 ± 14.54	351.83 ± 55.96	99.09 ± 1.29	11858.61 ± 179.03	1.43 ± 0.07[b]	2.93 ± 0.50	9180.05 ± 93.83[b]	0.77 ± 0.00[b]	2.96 ± 0.01[b]	76.31 ± 1.23
50BSF+	139.28 ± 16.02	351.89 ± 53.90	100.00 ± 0.00	11717.90 ± 926.73	1.42 ± 0.06[b]	2.90 ± 0.22	9137.45 ± 850.72[b]	0.78 ± 0.01[b]	2.93 ± 0.04[b]	77.35 ± 0.48
75BSF	135.54 ± 16.04	351.70 ± 54.39	100.00 ± 0.00	11888.85 ± 684.69	1.47 ± 0.04[b]	2.99 ± 0.29	9550.90 ± 728.32[b]	0.80 ± 0.02[b]	2.89 ± 0.05[b]	75.47 ± 3.24
75BSF+	135.05 ± 15.66	352.13 ± 54.52	99.09 ± 1.29	11774.41 ± 596.96	1.44 ± 0.01[b]	2.94 ± 0.38	9208.60 ± 395.98[b]	0.78 ± 0.01[b]	2.94 ± 0.02[b]	76.17 ± 0.15
100BSF	135.66 ± 14.72	354.64 ± 51.83	100.00 ± 0.00	12043.90 ± 126.10	1.48 ± 0.03[b]	3.03 ± 0.46	9377.55 ± 255.76[b]	0.78 ± 0.01[b]	2.93± 0.05[b]	76.77 ± 2.20
100BSF+	133.04 ± 14.84	354.70 ± 51.08	100.00 ± 0.00	12191.35 ± 716.51	1.47 ± 0.02[b]	3.01 ± 0.40	9559.00 ± 573.46[b]	0.78 ± 0.00[b]	2.92 ± 0.04[b]	75.56 ± 1.02
Model effects (_p-value_)										
P	0.765	0.055	0.737	0.071	0.018	0.834	0.018	0.007	0.001	0.066
GG	0.386	0.243	1.000	0.421	0.843	0.995	0.331	0.295	0.785	0.358
P*GG	0.487	0.782	0.382	0.950	0.971	0.999	0.781	0.155	0.362	0.754

p = 0.738 and F(4,5) = 5.00; p = 0.06, respectively), nor was mucosal layers thicknesses affected by the different feeds (Table 12). Observed rates of inflammatory cell infiltration of the *lamina propria* of the proximal intestine ranged from 0.25 to 0.88 (Figure 14A and B). This represents a low density of inflammatory cells and the difference between treatments was not significant (F(4,11) = 0.17; p = 0.947). The same scenario was observed in the distal intestine with infiltration rates ranging from 0 to 0.75 (F(4,30) = 0.003; p = 1.000) (Figure 14 A and B).

In both intestinal segments, enterocyte vacuolization was observed in the *lamina epithelialis*, but not influenced by dietary treatment. Low to moderate vacuolization was reported in the proximal intestine, scoring from 0.94 to 2.06 (F(4,30) = 0.003; p = 1.000) and low values from 0.19 to 0.94 (F(4,30) = 0.003; p = 1.000) were observed in the distal intestinal epithelium (Figure 15 A and B). Superficial observations of epithelium, suggested both intestinal segments exhibited normal goblet cell counts, which were also similar between treatments and between different parts of the intestine (Table 12). Also, the goblet cell filling did not vary between groups in each analyzed section, with moderate values observed for both proximal (from 1.9 to 2.4 (F(4,30) = 0.005; p = 1.000)) and distal (from 1.4 to 2.1 (F(4,13) = 0.18; p = 0.944)) tissues (Figure 16A and B). Regardless of the percentage of dietary BSF, all intestinal sections contained

Table 11 Organomatic indexes and processing yields of rainbow trout fed with the experimental diets. Values for VSI, HOG and SK-ON FIL are presented as means ± standard deviation (n=10). Values for HSI are presented as means ± standard deviation (n=2). P: Percentage of Insect Protein (0 (control), 25, 50, 75, 100); GG: Guar Gum (aditional GG); P*GG:Interaction of P with GG. VSI: Viscerossomatic index, HSI: Hepatossomatic index, HOG: Head-on, gutted, SK-ON FIL: Skin-on fillet.

Diet	VSI (%)	HSI (%)	HOG (%)	SK-ON FIL (%)
Control	15.29 ± 0.05	1.45 ± 0.01	84.71 ± 0.05	50.88 ± 0.16
Control+	16.38 ± 0.44	1.45 ± 0.01	83.62 ± 0.44	50.40 ± 0.31
25BSF	15.28 ± 0.66	1.85 ± 0.05	84.72 ± 0.66	50.88 ± 1.05
25BSF+	16.84 ± 0.77	1.57 ± 0.11	83.16 ± 0.77	50.41 ± 0.89
50BSF	16.19 ± 0.45	1.79 ± 0.19	83.81 ± 0.45	49.99 ± 0.75
50BSF+	15.80 ± 0.31	1.78 ± 0.43	84.20 ± 0.31	50.41 ± 0.52
75BSF	15.88 ± 0.74	1.68 ± 0.11	84.12 ± 0.74	51.50 ± 0.28
75BSF+	14.45 ± 2.05	1.87 ± 0.09	85.55 ± 2.05	51.63 ± 2.27
100BSF	15.09 ± 0.64	1.76 ± 0.24	84.91 ± 0.64	51.70 ± 0.89
100BSF+	15.08 ± 0.52	1.74 ± 0.27	84.91 ± 0.52	50.04 ± 0.59
Model effects (*p-value*)				
P	0.351	0.312	0.351	0.415
GG	0.221	0.576	0.221	0.634
P*GG	0.165	0.540	0.165	0.613

goblet cells stained purple by PAS-staining and vivid blue by AB-staining, indicating the presence of both neutral and acidic mucins. No differences were evident between treatments for in either tissue type (scored at 2.4 to 3 $(F_{(4,30)} = 0.21; p = 0.932)$, for proximal intestine and 2.6 to 3 $(F_{(4,18)} = 0.06; p = 0.934)$, for distal intestine) (Figure 17 A and B). No level of dietary BSF appeared to induce any significant alteration in rainbow trout intestine when compared with trout fed SPCs, and not did the inclusion of 0.3 % GG influence any of the evaluated histological parameters.
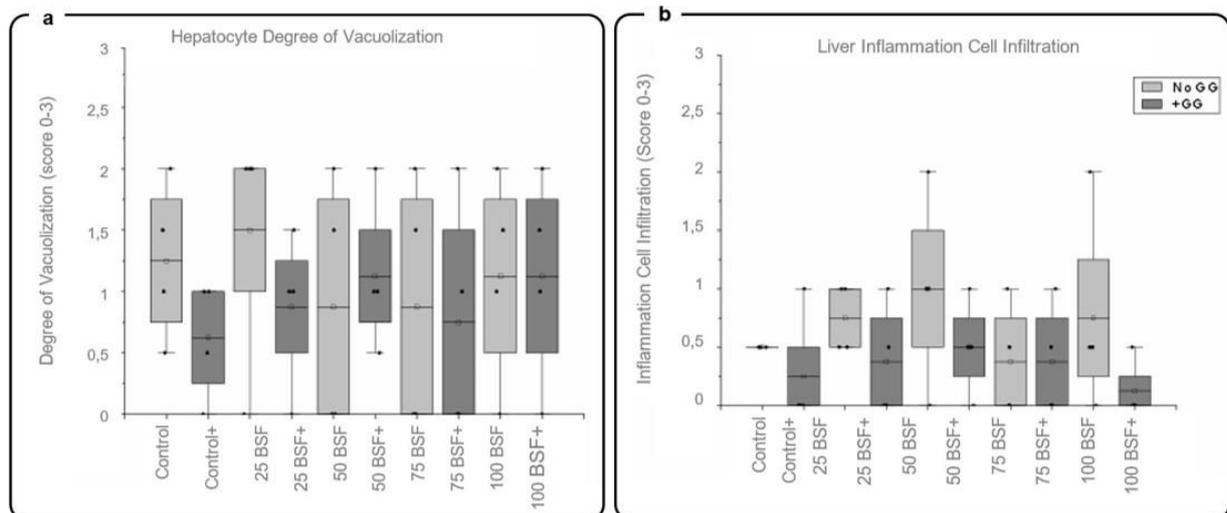
Figure 13 Examination of the liver of trout for signs of hepatocyte vacuole formation (A), and inflammatory reactions (B) of rainbow trout fed diets composed with increasing levels of *H. illucens* larval meal.
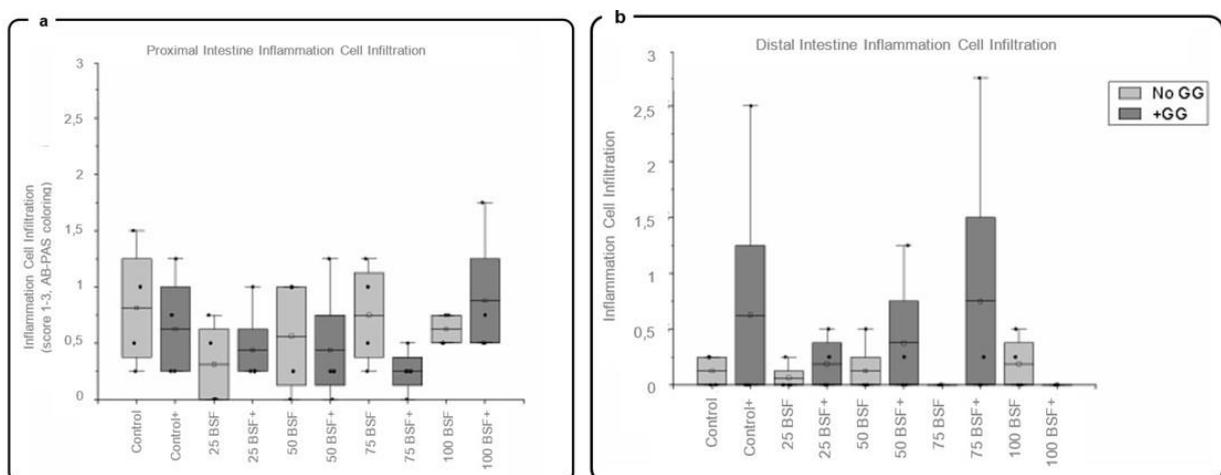
Figure 14 Examination of the proximal and distal intestine of trout for signs of inflammatory reactions (A and B) of rainbow trout fed diets composed with increasing levels of *H. illucens* larval meal.

71
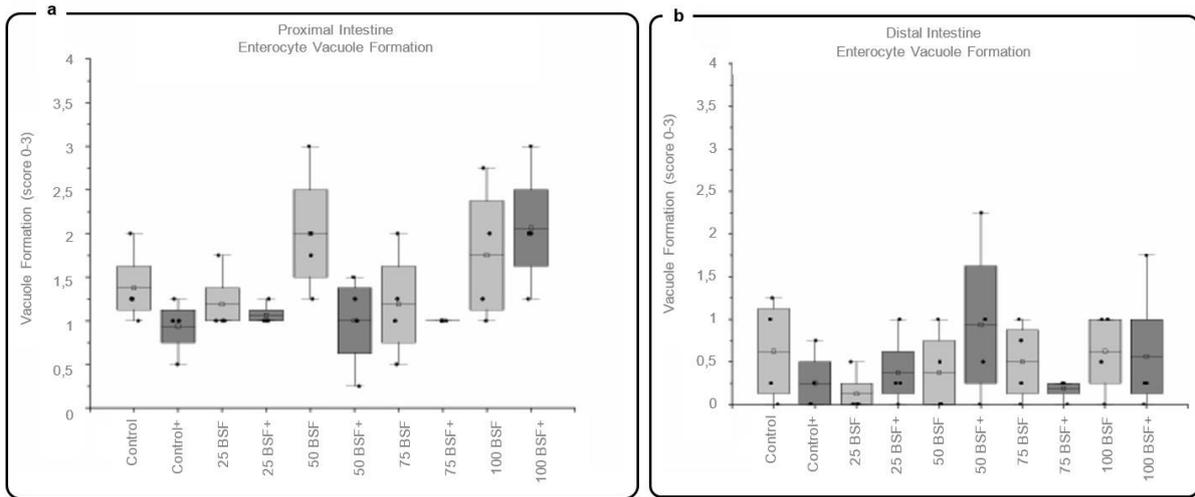
Figure 15 Examination of the proximal and distal intestine of trout for signs of enterocyte vacuole formation (A and B) of rainbow trout fed diets composed with increasing levels of *H. illucens* larval meal.
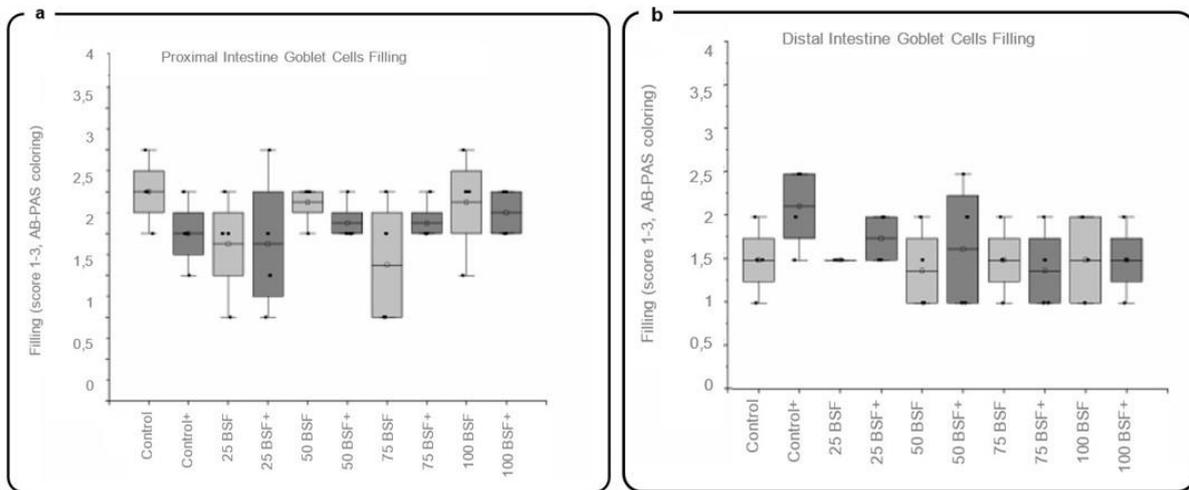
Figure 16 Examination of the proximal and distal intestine of trout for signs of goblet cells mucus filling (A and B) of rainbow trout fed diets composed with increasing levels of *H. illucens* larval meal.
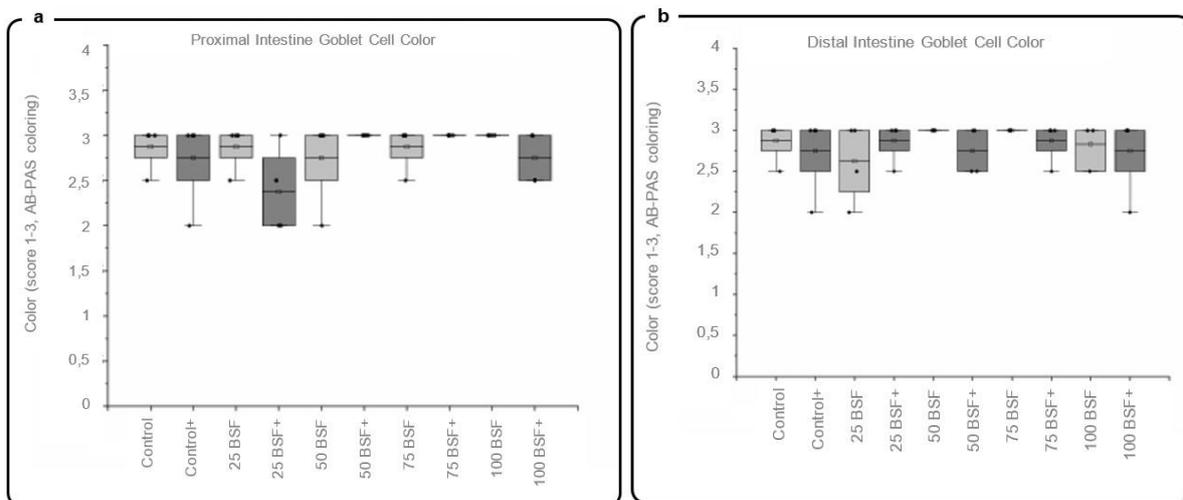
Figure 17 Examination of the proximal and distal intestine of trout for signs of goblet cells mucin color (A and B) of rainbow trout fed diets composed with increasing levels of *H. illucens* larval meal.

Table 12 Effects of dietary BSFML inclusion in rainbow trout's proximal and distal intestine morphology and mucin-producing goblet cells.

Diet	Diameter (µm)	Goblet cells amount (10x100 µm)	Thickness *str. granulosum* (µm)	Thickness *str. compactum* (µm)
Proximal intestine				
Control	3175.58 ± 414.00	4.81 ± 0.52	30.95 ± 11.04	23.45 ± 9.28
Control+	4144.00 ± 534.43	3.93 ± 1.13	28.83 ± 8 08	20.48 ± 3.41
25BSF	4398.63 ± 917.01	4.94 ± 0.52	42.13 ± 14.50	22.43 ± 9.09
25BSF+	3781.83 ± 234.51	4.69 ± 0.63	38.7 ± 13.91	26.00± 12.36
50BSF	3546.67 ± 695.95	5.63 ± 1.30	38.00 ± 11.64	21.80± 7.00
50BSF+	3897.25 ± 569.79	4.19 ± 0.94	38.45 ± 13.06	21.20 ± 8.85
75BSF	3466.66 ± 465.04	5.19 ± 1.55	29.53 ± 6.54	16.40 ± 3.58
75BSF+	4264.17 ± 361.64	4.56 ± 1.07	35.13 ± 20.02	18.38 ± 5.23
100BSF	3619.83 ± 308.93	4.50 ± 0.35	35.43 ± 8.17	18.90 ± 2.22
100BSF+	3563.7 ± 414.00	5.50 ± 1.06	40.88 ± 18.03	16.68 ± 5.65
Model effects (*p-value*)				
P	0.738	0.812	0.492	0.142
GG	0.0795	0.289	0.820	0.462
P*GG	0.311	0.315	0.928	0.626
Distal intestine				
Control	4544.5 ± 260.22	4.86 ± 1.16	36.75 ± 10.78	16.68 ± 3.52
Control+	5251.25 ± 1125.36	4.94 ± 1.13	27.63 ± 10 12	18.03 ± 3.15
25BSF	6145.13 ± 503.88	4.00 ± 0.74	29.78 ± 10.99	15.98 ± 9.26
25BSF+	6587.00 ± 84.15	4.25 ± 1.32	32.35 ± 10.94	17.00 ± 5.70
50BSF	5413.63 ± 826.85	5.31 ± 1.30	31.08 ± 6.84	18.65 ± 2.42
50BSF+	5587.17 ± 238.86	5.00 ± 1.40	40.00 ± 17.75	16.10 ± 4.50
75BSF	4541.5 ± 2368.10	4.31 ± 1.14	43.38 ± 14.21	18.90 ± 7.64
75BSF+	5347.00 ± 187.26	5.06 ± 1.42	33.95 ± 12.40	14.40 ± 2.87
100BSF	4327.33 ± 1226.35	3.88 ± 0.95	39.70 ± 14.60	23.13 ± 7.52
100BSF+	5052.25 ± 235.11	4.88 ± 1.16	26.85 ± 2.71	19.03 ± 7.59
Model effects (*p-value*)				
P	0.061	0.435	0.645	0.609
GG	0.392	0.940	0.260	0.762
P*GG	0.919	0.786	0.311	0.794

Expression profiling of the intestine. The complete substitution of dietary SPC with BSF plus 0.3 % GG yielded only a small effect on the transcriptome of proximal intestine in experimental fish (Figure 18A), with a 1.5 fold upregulation of Agilent feature A_05_P448532 predicted in the 100BSF+ group compared to the controls (with q = 0.036). This feature was identified as *Oncorhynchus mykiss* gene LOC110491086 (NCBI gene ID: 110491086) which corresponds with a human orthologue encoding disks large associated protein 4 (DLGAP4). The elevated transcript concentration in the proximal intestine of trout fed 100BSF+ was confirmed via RT qPCR revealing a

1.96 fold greater expression (p = 0.047) compared with fish fed the control diet (Figure 18B).
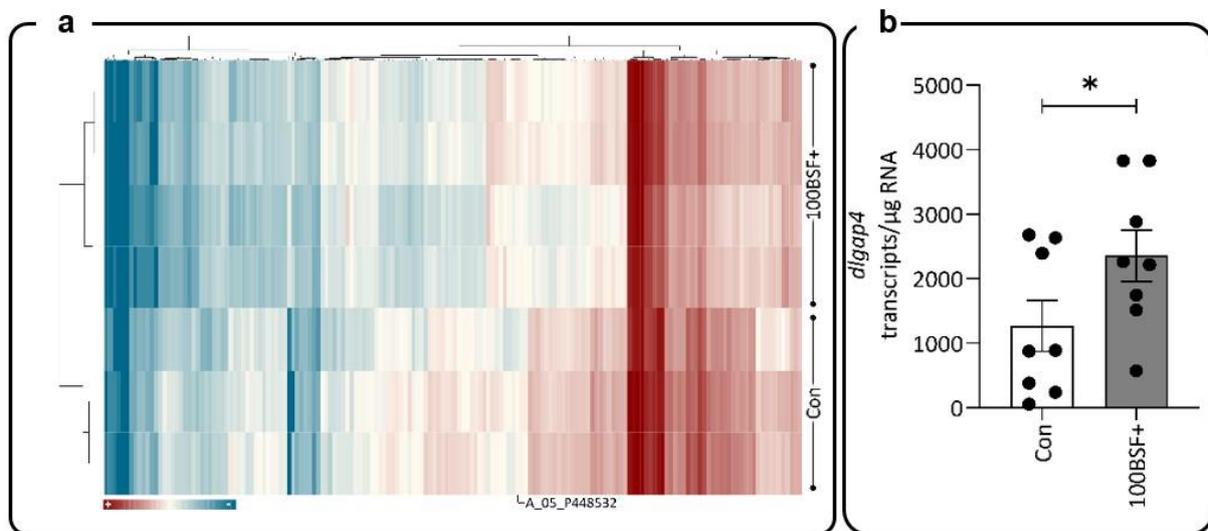
Figure 18 Transcriptomic analysis of proximal-intestine samples from rainbow trout after feeding 100 % BSF+ or a control diet. (A) Hierarchical clustering of log10-transformed transcript abundance of differentially expressed genes (with p < 0.001) from the microarray-based comparison of 100 % BSF+ group (upper panel) versus control group (Control, lower panel). High and low gene expression intensities are represented by red and blue colors, respectively. Note that only one gene (dlgap4; Agilent ID: A_05_P448532) passed the threshold of q < 0.05. (B) Bar plot illustrating the dlgap4 transcript abundance in trout fed 100 % BSF+ group (grey bar) versus control trout (white bar) as assessed with RT-qPCR. Bars represent mean values (± standard error of the mean); dots represent individual transcript numbers; the asterisk indicates p < 0.05.

4.5. Discussion

The present study investigated the effects of replacing SPCs with graded inclusions of BSFLM on the growth performance, feed utilization, and health of rainbow trout. To the best of our knowledge, no other study has yet investigated the total replacement of SPCs, which still is the main protein source in low FM diets for rainbow trout.

From the beginning of the experiment, all fish, regardless of treatment, foraged enthusiastically for all supplied feed, suggesting that insect content had no adverse effect on the palatability and acceptance of feeds. This conclusion was further evidenced by the increased feed consumption of fish fed BSFLM. Similar behavior has been previously observed for both rainbow trout and Atlantic salmon (*Salmo salar*) fed dietary BSF (Belghit et al., 2018; Cardinaletti et al., 2022). Replacing up to 100 % of SPC content with BSFLM did not result in any notable effect on overall fish performance. In fact, fish on all treatments demonstrated good growth and feed utilization. Additionally, the presence of 0.3 % GG did not impact any of the measured parameters, confirming the findings of Schumann et al. (2022).

After 10 weeks, there were no observable negative effects on fish growth, of even total replacement of dietary SPC with BSF. On the contrary, all fish fed some proportion of BSF in their diets exhibited enhanced growth compared to those on the commercial reference diet containing only SPC as a protein source. This suggests that even at just 25 % replacement BSFLM can have a positive effect on rainbow trout growth rates, but this effect was not further improved by higher levels of BSF inclusion. This suggests that insect content might provide mitigation of some nutritional deficiencies of SPC, such as a deficiency in some essential components, which appears can be fully compensated even by the lowest BSFLM level (Dietz and Liebert, 2018). Studies have already demonstrated the potential of BSF to replace FM in salmonid diets (up to 50 %) not only without adversely affecting growth performance (Caimi et al., 2021; Renna et al., 2017), but actually improving SGR (Weththasinghe et al., 2021b). Our results are not directly comparable with the previous literature due to differences in the used feed materials, principally the substitution of high concentrated SBM ingredients in low FM diets. Comparable growth was previously reported by Hossain et al. (2021) for rainbow trout fed on FM and soy based diets with low levels of BSFLM. However, contrary to our results, fish fed with higher levels of BSF exhibited weaker performance. Compared to SBM, SPC is characterized by optimal digestibility and low ANFs content (Kaushik et al., 1995; Li et al., 2015), possibly explaining the contrary outcomes of these studies, through the reduction in dietary SPC in Hossain's study, while substantial levels of SBM were maintained. Furthermore, variances in BSFLM quality related to insect life stage, diet, and processing methods could contribute to such discrepancies, as they can distinctly influence the fatty acid, vitamin and mineral content of the resulting product (Oonincx and Finke, 2021).

Previous studies have reported improved SGRs in fish fed with insects, often linked to higher feed intakes (Weththasinghe et al., 2021b). In our study, all experimental groups exhibited good feed uptake, although fish consumed significantly more feed containing BSFLM compared to both insect free control groups. This may be related to the levels of FO in the experimental diets, which increased proportionally with the inclusion of insects. The use of FO in aquafeeds is known to significantly enhance palatability to rainbow trout, which as a carnivorous species have a preference for diets rich in n-3 long-chain polyunsaturated fatty acids (ω-3 LC-PUFAs) (Roy et al., 2020). Despite their increased feed intake, fish fed with BSFLM exhibited reduced feed efficiency, in-

dicating a less efficient process for digestion (Davis and Hardy, 2022) than in the control groups. Recently, Randazzo et al. (2021b) reported that rainbow trout fed a diet containing 45 % BSF (60 % replacement level) and completely deprived of FM, exhibited better growth performance and feed utilization than those given a plant based diet, and similar or slightly better than those on an FM based diet. These results coincided with increased feed intake as the proportion of insects increased. Comparable trends in growth and feed efficiency were observed in our study when SPC was replaced by BSFLM, although an opposite effect on FCR was observed for the plant based reference diet, as mentioned before. These discrepancies can again be attributed to the use of SPC as the main reference protein source and the very low to non-existent levels of SBM in the present diets, supported by the significantly greater protein retention efficiency in groups fed the control diets.

In the present study, dry matter digestibility remained unaffected by dietary treatment, but there was a slight tendency for diets containing 50, 75, and 100 % BSFLM to more digestible than the insect free diets. Reduced digestibility in aquafeeds containing insects is generally associated with the presence of chitin in insect meal. Chitin is a complex natural polymer—consisting of β-1,4 inked subunits of N-acetyl D-glucosamine—which has already been shown to significantly lower the dry matter digestibility of rainbow trout feed when present in significant levels (*e.g.* 15.4 %, on a dry matter basis) and is directly negatively related to particle size with particles > 400 μm already recognized as having anti-nutritional effects (Eggink et al., 2022). The dry matter chitin content in BSF larvae, however is low, ranging from 1 9 % (Eggink and Dalsgaard, 2023; Meneguz et al., 2018) and species such as rainbow trout exhibit chitinolytic activity, making them not only capable of utilizing chitin but also of regulating exochitinase activity depending on its concentration in their diet (Eggink et al., 2022). Moreover, fish fed diets including 1 to 5 % chitin also exhibit improvements in intestinal microbiota and a gut microflora richer in chitinase producing bacteria (Askarian et al., 2012; Bruni et al., 2018; Huyben et al., 2019). Efficient digestion has already been observed in rainbow trout (Caimi et al., 2021), Atlantic salmon (Weththasinghe et al., 2021b), seabass (*Dicentrarchus labrax*) (Basto et al., 2020), gilthead seabream (*Sparus aurata*) (Moutinho et al., 2022) and barramundi (*Lates calcarifer*) (Le Boucher et al., 2024) fed with non dechitinized BSF with chitin levels ranging from 5 to 7 %, compared to fish fed with FM, with either neutral or positive effects on digestibility reported with increasing levels of insect content. Although not quantified in the present study, the chitin

content in the diets probably did not reach the threshold levels required to negatively impact digestibility. The observed tendency for a low digestibility in the control groups could also be attributed to the presence of 30 % SPC in the control diets. Despite SPCs having reduced levels of ANFs, they can still contain traces of soybean antigens like agglutinins, saponins, and phytic acid (Zhu et al., 2020). In addition, SPCs may also retain certain polysaccharides such as starch and insoluble fiber (Schumann et al., 2022), which could potentially reduce dry matter digestibility. Similar ADCs (over 75 % ADCDM) were observed in Atlantic salmon fed BSFLM as a replacement for SBM, although with significant improvements in the insect based diet compared to the soy based diet after a 16 week feeding trial (Fisher et al., 2020).

The relative size of liver and viscera, together with the proportion of edible flesh obtained from farmed fish after processing, are key indicators of efficient feed utilization leading to more profitable and sustainable production (Bugeon et al., 2010). The hepatosomatic index is also an indicator of metabolic function and energy reserves. Values presented in the published literature as indicators of proper liver function vary between 1 and 2 % (Iaconisi et al., 2018), while the viscerosomatic index reflects organoleptic health and fat deposition in the visceral cavity, which are largely influenced by the lipid content of the feed (Jobling et al., 1998). In the current trial, the insect protein treatments resulted in HSI and VSI values within the viable range for farmed rainbow trout, with no apparent dose effect. Even apparently small differences in processing yields can have a significant economic impact when long term cumulative quantitative effects are considered (Bugeon et al., 2010; Davidson et al., 2014). Organosomatic indices calculated in the present study point to no difference in processing yields (HOG and SK-ON FIL) by the inclusion of insect protein in the diet, compared to the reference SPC diet, which represents normal values for farmed rainbow trout (Davidson et al., 2014). In this respect, BSFLM could be a viable replacement for SPCs in commercial diets without compromising production economics.

As the gastrointestinal tract represents the first direct interaction with novel diets, histological examination of gut tissues is a routine component of nutritional trials, providing an integrative overview of organ integrity and functionality (Rašković et al., 2011; Wang et al., 2017). An inflammatory response is regarded as a primary indicator of negative effects (Miebach et al., 2023). In the current study histological examination did not reveal any severe alterations of liver tissue in terms of inflammatory cell infil-

tration. Low to moderate levels of cytoplasmic hepatocyte vacuolization were observed, but these were not linked to any dietary treatments. Previous studies have reported widely variable effects of insect diets on fish liver, with some reporting increased lipid accumulation to severe liver steatosis, with a linear increase of total saturated fatty acids (SFA), and associated decreases in PUFAs when insects were progressively incorporated into fish diets (Cardinaletti et al., 2019; Zarantoniello et al., 2020). Some, in line with our results, reported no effects when replacing FM or SBM with insect alternatives (Kumar et al., 2021; Melenchón et al., 2022), while others even report beneficial effects of BSFLM on lipid accumulation levels (Cardinaletti et al., 2022). In studies with Atlantic salmon (Belghit et al., 2018) and barramundi (Chaklader et al., 2021), authors also described a mitigating effect of BSFLM on liver lipid accumulation, hypothesizing that the significant amount of lauric acid in insect meals might prevent lipid deposition by stimulating its rapid oxidation. Such differences are probably related to the different nutritional composition of the experimental diets used.

Histological parameters were also evaluated in both proximal and distal sections of the intestine, revealing no evidence inflammatory responses linked to increasing levels of BSF. This accords with several previous studies showing an absence of intestinal inflammatory signs when BSFLMs were applied in teleost diets (Caimi et al., 2020; Elia et al., 2018; Lock et al., 2015; Miebach et al., 2023). The majority of marine and freshwater fish are planktivorous during their larval and juvenile life stages, and even species that become piscivores, herbivores or detritivores as adults may start their feeding activity with zooplanktivory, with insects as part of their natural diet. Carnivorous species like salmonids frequently prey on cladocerans, copepods and insect larvae as during their larval stage, while juvenile diets are dominated by larval, pupal or adult insects, before switching to fish dominated prey (Nunn et al., 2011). In addition, insects contain certain bioactive peptides and polysaccharides that have already been shown to reduce intestinal inflammation (Gasco et al., 2021), and partial inclusion of BSFLM in aquafeeds has shown to be beneficial in preventing SBM induced enteritis in rainbow trout (Kumar et al., 2021). Although we did not have access to information on the levels of specific dietary agents involved in the upregulation and pro-inflammatory responses present in the BSFLM composition (*e.g.*, pro inflammatory cytokines), these factors may have contributed to the intestinal health of the fish in our study (Gasco et al., 2021). No indications of enteritis were found, and dietary changes did not affect intes-

tinal macro morphology or goblet cell density. Additionally, besides no observable differences were found within the analyzed intestinal sections, a more pronounced enterocyte steatosis with moderate level of cell vacuolization was observed in the proximal intestine, whereas less evident in distal intestine. This phenomenon is known to be associated with high levels of plant inclusion in fish diets (Gu et al., 2013), and similar effects have previously been observed in both proximal and distal intestines when SPC was used as a replacement for FM in rainbow trout (Escaffre et al., 2007). In contrast to other studies, where BSF diets led to a reduction enterocyte vacuole formation, no such effect in rainbow trout's intestine was found (Y. Li et al., 2019; Weththasinghe et al., 2021b). An accumulation of lipids in the intestines of the control groups was to be expected, as plant ingredients are typically lower in phospholipids than FM, especially phosphatidylcholine, which plays a direct role in lipid transport across the intestinal mucosa (Krogdahl et al., 2020). However, the lack of changes in enterocyte condition in fish fed the BSF diets compared to controls was unexpected. Insects are reported to contain significant amounts of choline—the essential nutrient of phosphatidylcholine—previously been shown to prevent lipid accumulation in the proximal intestine of Atlantic salmon (Hansen et al., 2020; Krogdahl et al., 2020; Oonincx and Finke, 2021). The reasons for this lack of difference between fish fed with SPC and BSF remains unclear and a detailed comprehensive analysis of the used BSFLM should be considered in future investigations. Furthermore, no macroscopic signs of lipid malabsorption were observed among the analyzed fish, nor any other indication of bad health. Our results point to considerable potential for BSFLM as a viable replacement for SPC in salmonid diets. In all experimental groups, goblet cells showed a considerable degree of filling and a diversified mucin composition, indicating good intestinal functionality, without no excessive secretion of mucus into the intestinal lumen, which normally represents a defensive response to irritants, as part of the primary barrier against infection (Jung- Schroers et al., 2018). Variation in dietary levels of BSF did not influence the thickness of submucosal layers. These results suggest that rainbow trout can efficiently utilize BSFLM as a protein source without compromising liver and intestine health.

Corroborating the histology results, the transcriptomic analysis of intestinal tissue samples from rainbow trout revealed that only the dlgap4-gene was significantly upregulated in trout fed with 100 % BSFLM and 0.3 % GG compared with controls. Although the precise function of the teleost dlgap4 is unknown, the human DLGAP4-orthologue links scaffold proteins and associated signaling molecules to membrane bound ion

channels and receptors, especially glutamate receptors, and indirectly controls their activity (Rasmussen et al., 2017). The glutamatergic receptor activity of the interaction between microbiota and intestine can, in turn, influence the visceral sensitivity and motility of the gut, and alterations in glutamatergic transmission may contribute to localized pathogenesis (Baj et al., 2019). Previous studies consistently imply that partial substitution of FM with insect alternatives does not trigger the kind of inflammatory responses or physiological disorders seen in finfish fed on other substitute components with anti-nutritive properties, such as SBM (Krogdahl et al., 2015; Seibel et al., 2022). Moreover, the partial or complete substitution of FM by insect meal induces only low to modest changes in the expression of selected marker genes in defined gut segments of different aquaculture fishes, including gilthead sea bream (Carvalho et al., 2023; Piazzon et al., 2022) or Atlantic salmon (Li et al., 2020; Y. Li et al., 2019).

The demand for alternative feed ingredients to mitigate the aquaculture sector's demand for protein presents an opportunity for the insect industry (I.P.I.F.F., 2019). While currently contributing only a minimal part of salmonid diets (Aas et al., 2022a, 2022b), insects are already considered a reliable source for feed ingredients of aquafeeds. However, the question whether the insect industry can thrive in Europe remains unanswered. The production of insects still faces considerable challenges in Europe, making a large scale European insect industry at best a long term goal (Niyonsaba et al., 2023). The sustainable production of insect feeds, largely depends on to the possibility of using organic waste streams (van Huis, 2020), which are still not allowed in Europe, making the sector economically expensive and environmentally unstable in the near term (Veldkamp et al., 2022).

4.6. Conclusion

The present study supports the viability of black soldier fly larvae as a complete replacement for soy protein concentrate in trout feeds at an inclusion level of 40 % without affecting fish growth, feed utilization and dressing levels. Furthermore, even at high levels of dietary incorporation BSF did not negatively affect palatability or dry matter digestibility and there were no adverse effects on liver and intestine histology compared to fish on the SPC based control.

5. General discussion

5.1. Main findings

The first part (second chapter) of this thesis investigated how short-term weather changes and long-term global warming affect cold-water flow-through aquaculture and developed measures concerning how the sector could adapt to these challenges. It was found that current average water temperatures range between 9 and 17 °C with a maximum daily temperature increase of 3.7 °C. Thus, temperatures already exceed the recommended 17 °C on some days. However, with oxygenation, temperatures up to 19 °C are tolerable for rearing rainbow trout. In addition, occasional temperature increases in excess of 2 °C were recorded at all sites, with one site (B) showing a sudden increase of up to 5 °C following heavy rainfall combined with high summer temperatures. Due to the reduced oxygen solubility of water with increased temperatures, and the parallel increase in metabolic rates, it can be challenging to reach to such rapid temperature changes. Another problem associated with high temperatures is increased excretion and therefore dealing with metabolic end products. In particular, reduced dilution caused by reduced water flow due to long dry periods that can lead to an accumulation of *e.g.* NH_3 and CO_2, which can cause significant health problems within the fish. The effects of prognosed future temperature increases strongly depend on the thermal energy exchange between air temperature, solar radiation and incoming water temperature, as well as flow and shading. In the worst-case climate scenario, up to 77% of facilities in southern Germany are expected to be located in areas with suboptimal conditions, thus reducing production rates. Suggestions developed to mitigate future problems with water temperature and availability include shading of the facility as well as the use of a temporary recirculation system.

The second part of this thesis assessed the impact of cold-water flow-through aquaculture on climate change. Scenarios were modelled for fish diets with different inclusion levels of fishmeal and various levels of photovoltaic use to identify potential improvements. The results showed a significant effect of photovoltaic use to compensate CO_2 emissions. Without photovoltaics, 1.8 kg CO_2 per kg living weight of rainbow trout was released into the atmosphere, while a 100% coverage with photovoltaics would result in a compensation to 0.8 kg CO_2 per kg of trout produced. A reduction of almost 50% in CO_2 emissions could be achieved by using 62% fishmeal in the feed instead of 0% fishmeal. The results indicate that aquaculture is an effective way to produce meat

81

in an environmentally friendly manner, although room for improvement remains, particularly regarding feed and the use of renewable energy.

The LCA analysis clearly showed that feed is the main emission source within trout production. Consequently, the final part of this thesis concentrated on its improvement and investigated the use of insect meal as an alternative protein source in salmonid feed. In a dose-responsive trial using five different levels of insect meal protein in the feed (0, 25, 50, 75, 100%), rainbow trout showed an increased feed intake, which correlated with better growth in the test group being fed insect feed, while the control group receiving soy protein concentrate as the main dietary protein source showed a better protein retention and feed conversion ratio. In addition, results indicate that even a complete replacement with insect protein does not affect the physiological well-being of reared trout.

5.2. Climate change effects on aquaculture

Particularly in the context of ongoing climate change, reducing emissions is important in all sectors, not only aquaculture. If climate change leads to an increase in water temperature and/or a deterioration in water supply, this can severely limit domestic trout farming and affect the welfare of the fish (Alfonso et al., 2021; Myrick and Cech, 2004; Ros et al., 2022). Following such stressors, a neuroendocrine response occurs, releasing hormones and enzymes such as cortisol, altering metabolism or reducing the immune response to conserve energy for other life-sustaining activities (Tort, 2011; Wendelaar Bonga, 1997). When stress becomes chronic, cortisol binds to mineral corticoids and glucocorticoids, leading to either the inhibition or expression of immune-related genes (Tort, 2011). Gene expression then manifests as the third step of the stress response, whereby reduced growth and increased susceptibility to disease occur (Barton, 2002). In general, the fitness of trout is reduced. This will be a relevant and growing problem if future temperatures increase as projected, allowing certain pathogens to spread more easily and colonize new areas (Vezzulli et al., 2013). A current prominent example already mentioned in the second chapter of this thesis is proliferative kidney disease (PKD). The life cycle of the respective myxozoan parasite – *Tetracapsuloides bryosalmonae* – includes bryozoans and salmonids. In recent years, the parasite has continued to spread and produce spores in waters above 10 °C. Juvenile trout reared in waters below 15 °C develop no signs of disease or mortality, although the disease

will break out when temperatures increase. To date, the only way to prevent fatal infection is to keep water temperatures low or restrict it to fish that survived PKD in cold water (Ros et al., 2022).

Another emerging disease pathogen in salmonids is *Streptococcus phocae*, which was first found in seals suffering from pneumonia. In Chile, where the disease has rapidly spread, farmed Atlantic salmon are infected at temperatures above 15 °C. Infected fish show symptoms such as hemorrhages, abscesses, pericarditis and enlarged liver and spleen after a few days (Romalde et al., 2008). No evidence of the pathogen has yet been reported for Germany, although it could emerge in the future. According to the environmental model developed (Chapter 2), it will be increasingly difficult to maintain water temperatures below 15°C in the future. One crucial aspect here is the flow, which is significantly affected by ground- or freshwater availability from the receiving water. In case of a shortage of fresh water due to prolonged drought and increased evaporation, not only will temperature rise but also the dissolved contaminants in the husbandry water will increase and therefore become more difficult to deal with. In addition, this is linked to increased metabolism due to warmer temperatures, which increases excretion, especially of CO_2 and NH_3. When these two effects come together, harmful concentrations of more than 0.03 mg/L (NH_3) and 30 mg/L (CO_2) can quickly occur (Ebeling and Timmons, 2012). The rising concentrations in the water make it increasingly difficult for fish to excrete these substances. In the worst case, gill excretion might be inhibited. As a result, CO_2 and NH_3 would increase in the blood of the fish, leading to hypercapnia, acidosis, convulsions and high mortalities (Danley et al., 2007; Fromm and Gillette, 1968; Randall and Tsui, 2002).

Breeding more temperature- and toxicant-resistant strains could be part of a solution to rising temperatures and pollutants. Native trout could be crossed with strains from *e.g.* Australia (*O. mykiss* Walbaum) to produce animals that can better cope with both reduced flows and higher temperatures. More adapted animals that can better cope with higher temperatures are less likely to become stressed. Their metabolism would also be adapted to the higher temperatures, which could reduce the excretion of pollutants. It would also be possible to genetically engineer fish to require less oxygen, which would have a lasting effect on reducing the oxygen requirements of fish farms. However, as salmonids are cold-stenothermic fish with relatively low adaptations, this has only limited potential.

Another way to combat rising temperatures would be to reduce water heating. As the results in chapter 2 have shown, the only effective way of achieving this is by shading the system. Since solar radiation plays an important role in the heating of water bodies, a roof over the raceway already significantly reduces the heating of water. If the receiving water body on the farm site were also shaded, an additional effect of water temperature reduction could be achieved. Since the sun changes throughout the year and does not always fall at the same angle, it is not always possible to guarantee perfect shading by means of a roof. Fish farms that are already covered could go one step further and use special nets to cover the sides facing the sun to reduce the exposure of the tanks to the sun even when the angle of incidence is shallower. Farms that are financially constrained and therefore cannot afford a roof could use nets either like a roof or directly over their rearing systems, as shown in Naas and Müller-Belecke (2023).

5.3. Emission reduction in aquaculture

Research to date suggests that it will not be possible to halt the effects of climate change within the next few years (IPCC, 2018). Nevertheless, efforts are being made to reduce emissions in all industries (Jacob et al., 2020; Koričan et al., 2022; McAuliffe et al., 2016; Qiu et al., 2021), with aquaculture being no exception (Bujas et al., 2022; Hancz, 2022; Lieke et al., 2020; Samuel-Fitwi et al., 2013a). Aquaculture operations are generally considered to be producing a particularly valuable and environmentally friendly food (Gephart and Golden, 2022). However, given the growing demand for fish, every effort must be made to maintain or even improve this status while still promoting considerable production growth. Possibilities include changes in feed production and the use of more renewable energy.

The adaptation of feed holds particular interest due to its central role in fish farming. In Norway, according to Aas et al. (2022b) 90,000 tons of rainbow trout were produced in 2020. Almost 117,000 tons of ingredients were used to produce their fish feed, mainly from vegetables (71%), followed by marine ingredients (24%) (Aas et al., 2022b). In aquaculture, highly valued species such as salmonids require a well-balanced diet to ensure optimal growth and subsequent nutrient composition of the fillets (Colombo et al., 2018; FAO, 2022; Rollin et al., 2003). Traditionally, feed quality has been ensured by the use of fish meal and fish oil (FAO, 2022). However, marine resources are limited and sustainable management is often questioned – as discussed

in chapter 3 – meaning that alternatives must be found. Reviewing the recent state of research of potential protein alternatives, a variety of candidates are identified ranging from algae to animal by-products and insect meal (Albrektsen et al., 2022).

Insect meal is currently highly discussed. Although the literature suggests that insects – particularly black soldier flies and mealworms – can be produced cheaply and in a small space, there is little evidence available that insect meal are truly more sustainable (van Huis and Oonincx, 2017). The high amount of energy required during production to maintain stable temperatures and humidity as well as processes such as dry freezing produce relevant emissions (Stadtlander et al., 2021; van Huis and Oonincx, 2017) if renewable energy sources are not used exclusively. In addition, EU Regulation 2017/893 stipulates that insects used as feed component are not allowed to be harvested with organic waste ("Regulation - 2017/893 - EN - EUR-Lex," 2017), whereby this requirement increases the ecological footprint of insect proteins. Nevertheless, there are some promising studies showing that insect meal could be an adequate substitute for fish meal or soy as a protein source regarding fish growth, performance and health (Freccia et al., 2020; Sánchez-Muros et al., 2014; Stenberg et al., 2019). To date, studies have failed to show that higher levels of insect meal and oil have any noticeable negative effects on farmed fish. This is unsurprising given that insects are part of the natural diet of salmonids in particular. However, it is thought that a high level of chitin might lead to poorer feed utilization (Eggink et al., 2022; Ómarsson, 2018; Weththasinghe et al., 2022). This study found minimal differences in the FCR of diets containing insect protein. Whether insect proteins have a future in aquaculture remains to be seen. Current studies suggest that under certain conditions such as renewable energy for production, it would be possible to use insect proteins as a sustainable, resource-saving alternative (Le Féon et al., 2019; van Huis and Oonincx, 2017).

Reducing emissions by conserving oxygen will tend to cause greater problems in the future. As this study shows, temperatures will rise in the future and quickly exceed the optimum temperature range for salmonids, at least temporarily. In terms of food security, it is important to maintain production at temperatures above the natural temperature optimum through *e.g.* new breeds or increasing dissolved oxygen levels in the water. Studies on trout have shown that the optimum temperature during rearing is a maximum of 17 °C, although increasing oxygen concentrations enables production up to temperatures of 20 °C (Ebeling and Timmons, 2012; Jonsson et al., 2001; Myrick

and Cech, 2004). The supply of technical oxygen is also important to maintain a constant production level regardless of water temperature. In this context, it would be possible to rethink oxygen production. At present, oxygen is often purchased from large companies, requires a lot of energy to produce, and has to be transported between production and consumption sites. An alternative would be to use oxygen generators installed directly on the fish farm. Combined with the use of renewable energy, such as photovoltaic or small hydroelectric power plants directly on the fish farm, emissions could be reduced. Depending on the production scale, it should be possible to produce all or at least a (large) part of the oxygen used more sustainably, reducing not only farm emissions but also making it independent from fluctuating energy and oxygen prices.

However, as the use of renewable energy is highly dependent on the energy mix available in a country or the liquidity of the farm owner, many key factors need to be considered. Most importantly, converting a fish farm to self-generated electricity is both a cost and a practical challenge, although the environmental parameters must also be adequate. For example, the use of photovoltaics only makes sense if the fish farm receives sufficient sunlight or has enough surface area to use. The situation is similar for (mini) hydroelectric power plants, for which there must be enough space either at the water inlet or outlet of the raceway, and the flow should be sufficiently large to ensure a sufficient supply of self-generated electricity.

However, if these basic requirements are met, and if the fish farmer is able to handle investment costs, the generation of sufficient renewable electricity can also be used to exploit other technical innovations such as temporary filter systems to reduce emissions on the farm.

5.4 Tackling the climate crisis

In summary, climate change will result in some challenges for salmonid aquaculture. As the atmosphere warms, extreme weather events such as droughts and heavy rainfall will become more frequent, whereby the temperature change will also affect the spread of certain diseases. It is therefore important to quickly find solutions to mitigate temperature rise in aquaculture systems that are both preventative and can be tailored to the specific needs of individual farms in a cost-effective and space-saving way. As mentioned above, this could be achieved by either breeding more tolerant trout strains or reducing ambient temperatures by shading or fogging systems. Another important

goal is to reduce emissions from the aquaculture sector itself to continue to provide the most sustainable, healthy and protein-rich food possible. As this study shows, this can be effectively achieved through the use of alternative feed components and roofing facilities with photovoltaic systems. The latter solution in particular can be used in a variety of ways. It not only reduces the temperature of the holding water and the impact of predatory birds on the fish population but also reduces emissions from the plant. The energy of the photovoltaic system could be used to apply on-farm oxygen production, thus further reducing emissions. Another principle that has not been further analyzed in this study but holds significant potential in other research is the use of temporary recirculation. This can be particularly useful in situations of water scarcity and high concentrations of pollutants in the water if inlet water is sufficiently cool.

Publication list

Original publications integrated in this thesis:

Chapter 2

Wind T., Ros A., Chucholl C., Roch S., Schumann M., Schulz C., Brinker A. (2023): Inland aquaculture in a warming world, Science of the total Environment, Volume 934, 173275, https://doi.org/10.1016/j.scitotenv.2024.173275

Chapter 3

Wind T., Schumann M., Hofer S., Schulz C., Brinker A. (2022): Life cycle assessment of rainbow trout farming in the temperate climate zone based on the typical farm concept, Journal of Cleaner Production, Volume 380, Part 1, 134851, ISSN 0959-6526, https://doi.org/10.1016/j.jclepro.2022.134851.

Chapter 4

Sousa e Brito S., Wind T., Schumann M., Rebl A., Jung-Schroers V., Schulz C., Brinker A. (submitted): Black soldier fly (Hermetia illucens) as a potential alternative for soy protein concentrate in rainbow trout (Oncorhynchus mykiss) diets, Aquaculture

Oral presentations related to this thesis

04/2021: Transportation of living fish – optimization of relevant water quality parameters during commercial trout transport with and without aeration; Aquaculture Europe, 2020 Online

09/2021: Der Transport von lebenden Fischen – Optimierung wichtiger Wasserparameter beim Forellentransport, Brandenburger Fischereitag

06/2022: Lebenszyklusanalyse: Regenbogenforellen in der Aquakultur; Büsumer Fischtag

09/2022: Life cycle assessment of rainbow trout farming based on the typical farm concept; Aquaculture Europe, Rimini

10/2022: Forellenzucht und Klimawandel; Fachforum Forellenzucht, Engen

05/2023: Daten und Fakten zum Klimawandel; DLG Sitzung Aquakultur, Trostadt

Authorship and contributions

AB = PD Dr. Alexander Brinker; AR = Dr. Albert Ros; Are = Dr. Alexander Rebl; CS = Prof. Dr. Carsten Schulz; MS = Dr. Mark Schumann; SH = Stephan Hofer; SR = Dr. Samuel Roch; SSB = Sara Sousa e Brito; TW = Tamara Wind

Chapter 2 Cold water aquaculture in a warming world

TW: Conceptualization, Data collection, Illustration, Statistical analysis, Initial draft.
AR: Conceptualization, Illustration, Statistical analysis, Review and Editing.
CC: Statistical analysis, Review and Editing, Illustration.
SR: Illustration
MS: Conceptualization, Data validation, Review and Editing, Supervision
CS: Review and Editing, Supervision.
AB: Conceptualization, Design evaluation, Data validation, Review and Editing, Supervision

Chapter 3 Life cycle assessment of rainbow trout farming in the temperate climate zone based on the typical farm concept

TW: Conceptualization, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Visualization.
MS: Conceptualization, Verification, Investigation, Resources, Writing –review and editing.
SH: Investigation.
CS: Writing – Review and Editing, Supervision.
AB: Conceptualization, Validation, Investigation, Writing – Review and Editing, Supervision.

Chapter 4 – Performance and physiological consequences of completely replacing soy protein in rainbow trout (*Oncorhynchus mykiss*) diets with black soldier fly (*Hermetia illucens*) larval meal

TW: Study conception and design, Data collection, Draft manuscript preparation, Illustrations (Figures), Editing and reviewing

SSB: Study conception and design, Data collection, Statistical analysis and interpretation of results, Draft manuscript preparation, Illustrations (Tables), Editing and reviewing

AR: Transcriptomic analysis, Statistical analysis and interpretation of results (transcriptomics), Editing and reviewing

DK: Transcriptomic analysis, Editing and reviewing

VJS: Histological analysis, Editing and reviewing

MS: Study conception and design, Editing and reviewing

CS: Editing and reviewing

AB: Study conception and design, Statistical analysis and interpretation of results (Fish performance and histology), Editing and reviewing

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