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DIPLOMARBEIT

Titel der Diplomarbeit

„Effect of aquaculture on essential nutrients and potential contaminants in benthic organisms and fish of pre-alpine streams“

Verfasserin

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ich bin zu der natur gegangen
die luft ein demantbrunnen ist
und hier, von weiden überhangen,
ernt ich die morchel, den bovist.

und nennt mich auch der müde städter
versponnen, spinner, später spund,
so ists mir gleich, potz regenwetter,
ich liebe meinen wiesengrund.

die welt ist immer noch beim alten,
der fungus wächst, wenn regen rauscht,
mirakel der naturgewalten,
ich hab es oft und gern belauscht.

(h.c. artmann – am teich. aus meiner botanisiertrommel)

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1. Einleitung

Weltweit ist ein starkes Wachstum im Bereich der kontrollierten Aufzucht von Speisefischen in Aquakulturanlagen zu verzeichnen. Der Beitrag, den die in Aquakultur produzierten Fische, Krebs- und Weichtiere an der weltweiten Fischproduktion hatten, lag laut der Ernährungs- und Landwirtschaftsorganisation der Vereinten Nationen (FAO) im Jahr 2000 bei 3,9%. Im Jahr 2007 lag der Anteil bereits bei 27,3 %. International nahm dieser Sektor damit im Durchschnitt um 9,2 % pro Jahr zu, während die Fangfischerei lediglich um 1,4 % pro Jahr wuchs. Die FAO sieht in der kommerziellen Fischzucht die einzige Möglichkeit, den weltweit wachsenden Bedarf an Fischprodukten zu decken. Die kommerzielle Fischzucht weist seit 25 Jahren die größten Wachstumsraten innerhalb der Nahrungsmittelbranche auf – sie wächst somit schneller als alle anderen Tier produzierenden Sektoren in der Land- und Fischereiwirtschaft. Bereits heute werden der FAO zufolge 48 Mio. t Fisch pro Jahr verzehrt, davon stammen rund 45 Prozent aus Aquakultur. (Vgl. fao.org).

Obwohl Österreich laut Statistik Austria (2008) global gesehen nur einen verschwindend kleinen Anteil an Speise- und Besatzfisch zur Fischproduktion beiträgt, mindert dies nicht die regionale Wichtigkeit dieses Nahrungsmittels. In Österreich werden in ca. 190 Teichanlagen Karpfen gezüchtet und ca. 232 Anlagen produzieren Forellen (siehe Abb.1). Im Jahr werden insgesamt rund 3300 Tonnen produziert, davon sind rund 2400 Tonnen Speisefische und rund 900 Tonnen Besatzfische. Die Hauptarten sind Forellen- (ca. 2000 t) und Karpfenartige (ca. 770 t). (Vgl. lebensministerium.at)

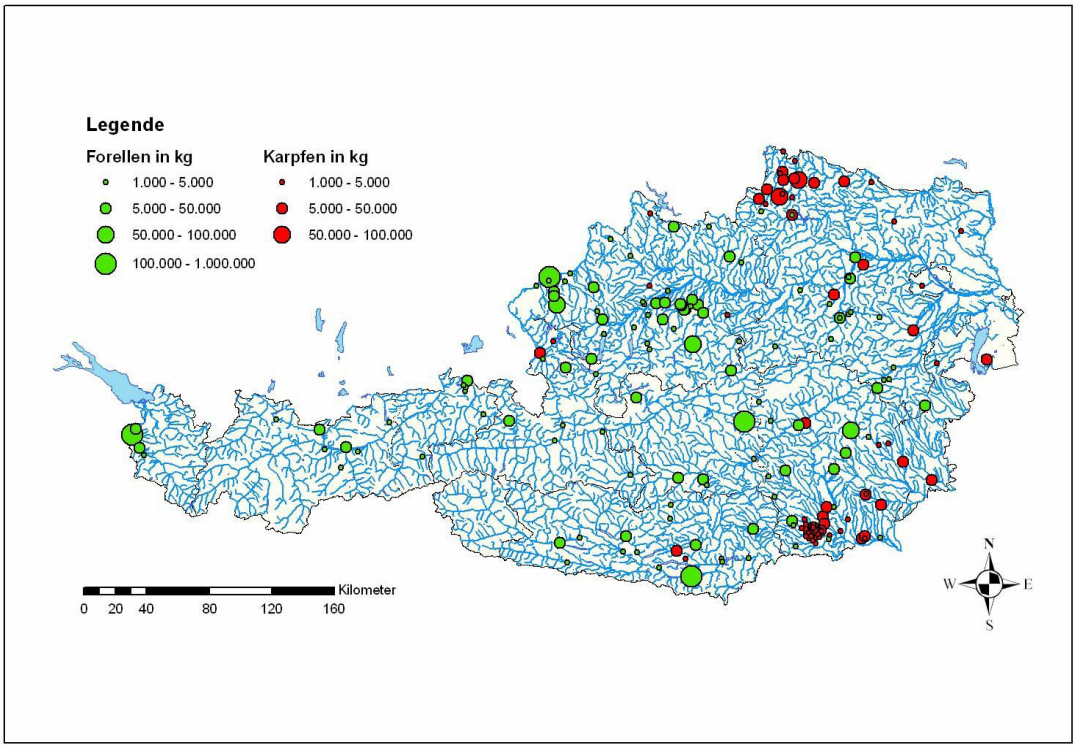

Abb.1: Lage der Aquakulturbetriebe ein Österreich und deren Forellen- bzw. Karpfenproduktion in kg. (Quelle: ec.europa.eu)

Die in Fischen enthaltenen Omega-3-Fettsäuren sind in aller Munde. Zahlreiche wissenschaftliche Untersuchungen der letzten Jahre sowie die stets steigende mediale Aufmerksamkeit haben den Fisch als die Hauptquelle für die menschliche Gesundheit, wichtigen und essentiellen Fettsäuren in den Blickpunkt gerückt. Bei regelmäßigem Verzehr können ua. Ablagerungen und Verengungen der Blutgefäße vermieden und somit das Risiko auf Herz-Kreislauf- sowie neurologische Erkrankungen reduziert werden (Dewailly et al., 2007). Vor allem während der Schwangerschaft und Stillzeit soll sich eine erhöhte Aufnahme dieser Fettsäuren zudem positiv auf die Gehirnentwicklung des Foetus und des Säuglings auswirken (Santerre, 2010).

Die Fettsäuren der Fische spielen aber nicht nur für den Menschen als gesunde Nahrungsergänzung eine große Rolle. In Form von Speicherlipiden versorgen sie auch den Fisch mit Energie, Strukturlipide tragen zum Aufbau von Zellwänden bei, sie sind wichtige Komponenten in Hormonen und stellen die Quelle für ungesättigte essentielle Fettsäuren (EFAs), welche nicht de novo synthetisiert werden und müssen folglich mittels der Nahrung aufgenommen werden müssen. Die wichtigsten und bekanntesten Vertreter der Omega-3

Fettäuren sind Alpha-Linolensäure (ALA), Eicosapentaensäure (EPA) und Docosahexaensäure (DHA).

Aquakulturanlagen können aber auch negative Effekte auf Fische sowie ganze aquatische Systeme, die durch den Wasserrücklauf der Aufzuchtbecken direkt betroffen sind, inkludieren. Darunter fällt sowohl die potentielle Kontamination der Organismen durch Schwermetalle und organischer Umweltchemikalien als auch die Erhöhung des Nährstoffangebots (zB. Lipide) in Flüssen und Bächen. Gewässerbelastungen (Emissionen) aus Fischzuchten umfassen demzufolge Futtermittelreste, welche nicht durch Nahrung aufgenommen werden, Ausscheidungsprodukte in partikulärer oder gelöster Form und Chemikalien, die zur Medikation und Desinfektion eingesetzt werden. Diese Stoffe können in aquatischen Organismen und folglich im Menschen bioakkumulieren, worunter man die Anreicherung bestimmter Stoffe von einer trophischen Ebene zur nächsten versteht. Bioakkumulation, als ökotoxikologischer Überbegriff, ist das Resultat zweier unterschiedlicher Prozesse. Zum einen können diese Stoffe a) aus dem umgebenden Wasser extrahiert werden, b) zum anderen werden sie über die Verdauung der Nahrung aufgenommen. Von Biokonzentration und Biomagnifikation wird deshalb dezidiert dann gesprochen, wenn das umgebende Wasser bzw. die Nahrung die Quelle der Stoffe ist.

Für ein fundiertes Verständnis von Bioakkumulationsprozessen ist eine Auseinandersetzung mit Trophie-Konzepten bzw. Nahrungsnetzen in Ökosystemen unerlässlich. Ein historischer Abriss der Erforschung von trophischen Beziehungen sowie die ökologischen Eckpfeiler sollen im nächsten Abschnitt kurz dargestellt werden:

Lebensgemeinschaften sind durch ständige Interaktionen sowie durch Stoff- und Energieflüsse zwischen den einzelnen Spezies gekennzeichnet. Der britische Zoologe und Ökologe Charles S. Elton (1927) erkannte Ende der zwanziger Jahre, dass das Fressen und Gefressenwerden eine fortlaufende Nahrungskette bildet. Auf ihn geht das Konzept der Elton'schen Zahlenpyramide zurück, die die Individuenzahlen der einzelnen trophischen Ebenen angibt. Sie besagt, dass der Zusammenhang zwischen der vorhandenen Nahrung und den Lebensgemeinschaften durch die Größe und die Anzahl der einzelnen Arten in einem

Ökosystem bestimmt wird. In anderen Worten: je kleiner die Art, desto höher ihre Abundanz und je größer, desto geringer ist die Häufigkeit im Ökosystem.

Lindemann legte mit seinem tropho-dynamischen Konzept (1942) einen weiteren Meilenstein in der Erforschung der Nahrungsketten. Es besagt, dass der Grundprozess der trophischen Dynamik im Transfer von Energie zwischen unterschiedlichen Teilen eines Ökosystems liegt. An der Basis stehen die Effizienz der Absorption von Sonnenenergie, der Prozess der Photosynthese durch Pflanzen, so genannte Produzenten, und ihre Umwandlung in andere chemische Energieformen. Obwohl Pflanzen einen Großteil dieser Energie für katabolische Prozesse verwenden, wird ein Teil an organischer Substanz akkumuliert und an heterotrophe Organismen, Konsumenten, weitergegeben. G. E. Hutchinson, der Vorreiter der modernen Limnologie, unterlegte das Konzept der trophischen Dynamik, indem er den unidirektionalen Fluss der Energie und deren kontinuierliche Abnahme von einer trophischen Ebene zur nächsten in limnischen Ökosystemen beschrieb. Die Produktion aufeinander folgender trophischer Ebenen nimmt demzufolge stark ab. Die Zahl der in einem Ökosystem möglichen trophischen Ebenen ist also von der Höhe der Primärproduktion abhängig, eine Nahrungskette kann folglich nicht unendlich lang sein (vgl. Lampert & Sommer, 1999). Die fortschreitende Forschung an den Strukturen von Lebensgemeinschaften und deren interne Energietransfers ließen bald das komplexere Bild eines Nahrungsnetzes an die Stelle der einfacheren Nahrungskette treten. E. P. Odum (1968, 1969) versuchte in seinen Arbeiten die Komplexität dieser Nahrungsnetzstrukturen und die daran gekoppelten Energieflüsse zu beschreiben. Er bekräftigte zudem die Wichtigkeit des Detritus als Energiequelle in Lebensgemeinschaften und beschäftigte sich mit biogeochemischen Nährstoffkreisläufen.

Laut dem Zoologen R.T. Paine (1980) sind Nahrungsnetze idealisierte Bilder von komplexen trophischen Mustern, welche sich je nach geographischer Lage oder Saisonalität ändern können. Er fokussierte seine Forschung auf die Interaktionen zwischen den einzelnen Arten einer Lebensgemeinschaft und prägte die Begriffe des „cross-linkage“ und der „linkage strength“. Die Strenge oder Wichtigkeit eines trophischen Zusammenhanges kann nicht für alle Mitglieder äquivalent zusammengefasst werden. Das Verschwinden einer weniger stark interagierenden Art führt zu wenig bis keiner Veränderung in der Struktur der Nahrungsnetze, starke Interakteure hingegen verursachen wahrnehmbare Veränderungen. Laterale Vernetzungen (Cross-linkage) zwischen einzelnen Kompetitoren unterschiedlicher

trophischer Ebenen können bei Ressourcen- oder Raumlimitation vorkommen, sollen aber nicht als trophische Bindeglieder angesehen werden.

Seit den 80ern hat sich das Wissen über terrestrische als auch aquatische Nahrungsnetze stetig gesteigert. Stabile Isotope, vor allem von Stickstoff und Kohlenstoff, wurden unter anderem als ein neues Hilfsmittel, um die Struktur von Nahrungsnetzen in aquatischen Ökosystemen zu erforschen, eingesetzt. Diese Isotope werden auf charakteristische Weise angereichert, wenn organisches Material von einem trophischen Level zur anderen weitergegeben wird (Harrod, 2006). Aufgrund der Isotopensignatur eines Organismus lässt sich die Herkunft der Nahrungsressourcen ermitteln; zudem sind sie nützliche Indikatoren von Energieflüssen (Peterson and Fry, 1987).

Die wissenschaftliche Erforschung der Nahrungsnetze in den darauffolgenden Jahren ist durch die Abwendung von einer holistischen Betrachtungsweise und einer separaten Analyse lentischer und lotischer Systeme zu beschreiben. So wurden große Arbeiten, lentische Systeme betreffend, geleistet. Van der Zanden (2006) konnte zeigen, dass benthische Energie- und Nahrungsquellen zur Produktion in höheren trophischen Levels in Seen beitragen. Carpenter (2005) hingegen erarbeitete die Wichtigkeit des Beitrags von terrestrischem, allochthonem Material zu Seenahrungsnetzen. Im Gegensatz zu stehenden Gewässern sind bei Flüssen und Bächen die einseitig gerichtete Strömung, stromabwärts führende Stoffspiralen und die Fließgeschwindigkeit die entscheidenden Parameter. Deren Einfluss auf Flussnahrungsnetze wurde u.a. von Power (2002) untersucht. In ihrer Pionierarbeit „Foodwebs in river networks“ erörtert sie die Interaktionen einzelner Arten untereinander, die Vorhersagekraft von Energieflüssen auf die Interaktionsstärke, die Abhängigkeit der Nahrungsnetze von räumlichen Energie- und Nährstoffquellen sowie die Verweildauer und den Transport der für Nahrungsnetzen notwendigen Stoffe. Thorp and DeLong (2008) beschäftigten sich mit den Veränderungen der Nahrungsressourcen für Flussorganismen entlang des longitudinalen Gradienten lotischer Ökosysteme. In einer Anlehnung an das River Continuum Concept (Vannote et al., 1980) erarbeiteten sie die Wichtigkeit des allochthonen Eintrags von organischem Material (Makrophyten, Blätter) als Energiequelle und deren flussabwärts gerichteten Transport.

Im Rahmen meiner Diplomarbeit habe ich den wissenschaftlichen Fokus auf die unterschiedlichen Fettsäure- und Schwermetallkonzentrationen im Muskelgewebe der Regenbogenforellen (*Oncorynchus mykiss*), Bachforellen (*Salmo trutta fario*) und des Eismeersaiblings (*Salvelinus lepeschini*), alle drei aus der Familie der Salmoniden, und deren potentielle Nahrung (Fischpellets, Makrozoobenthos und Phytobenthos) in Aquakulturanlagen und Bächen gelegt. Die Beprobungen fanden in vier Voralpen-Bächen und nebenliegenden Aquakulturanlagen in Niederösterreich statt.

Folgende Fragestellungen wurden behandelt:

- Wie sehen die Fettsäureprofile der Fische und ihrer potentiellen Nahrung, Makrozoobenthos in den Bächen und Fischpellets in den Aquakulturanlagen aus?
- Wie hoch ist die Schwermetallbelastung der Salmoniden sowie des Futters und wie setzt sie sich zusammen? Liegt die Belastung über den Richtwerten der Lebensmittelverordnung des Umweltbundesamts (UBA) sowie der Weltgesundheitsbehörde (WHO)?
- Welches Bioakkumulationsverhalten von PUFA (Polyunsaturated Fatty Acids) kann beobachtet werden?
- Welche Schwermetalle werden im Muskelgewebe der Fische bioakkumuliert?
- Wie unterscheiden sich die Nahrungsnetze dieser zwei aquatischen Lebensräume?
- Wie wirken Aquakulturanlagen auf Nahrungsqualität (essentielle Fettsäuren und potentielle Schadstoffe) des Futters im Vorfluter?

Um diese Fragen beantworten zu können wurden Lipidextraktionen am Wassercluster Lunz, stabile Isotopenanalysen an der Universität Wien und Schwermetallaufschlüsse am Umweltbundesamt durchgeführt.

2. First manuscript for submission

2.1. Abstract

Lipids, heavy metals, and stable carbon and nitrogen isotopes (SI) were examined in freshwater salmonids and their potential diets (macrozoobenthos and fishpellets) of 4 pre-alpine streams and aquacultures in Lower Austria. The aim of this study was to investigate (i) trophic position of fish and diet in streams and aquacultures, ii) comparing fatty acid and heavy metal profiles and their bioaccumulation patterns of the two aquatic habitats and to (iii) examine a potential effect of aquacultures in terms of nutrients and contaminants through the water discharge on nearby stream food webs.

Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) revealed different SI signatures for fish in aquacultures (-21.98 ± 0.17‰; 10.75 ± 0.67‰) and streams (-29.12 ± 0.40‰; 6.13 ± 1.02‰, respectively). Fractionation factors clearly showed that salmonids of aquacultures fed on pellets ($\Delta^{15}N$ 4.3‰, $\Delta^{13}C$ 0.55 ‰), whereas fish from streams fed on benthic invertebrates and other, not identified, diets ($\Delta^{15}N$ 4.5‰, $\Delta^{13}C$ 3.4‰). Results of dorsal muscle tissues analysis showed that the physiologically required omega-3 PUFA, docosahexaenoic acid (DHA; 22:6n−3) and the potentially toxic heavy metal mercury (Hg) were both mostly retained in all fish of all ecosystems. There were no significant differences of total lipid concentrations between AC and streams ($p=0.5$). In fish, arachidonic acid (ARA; 20: 4n-6; $p<0.001$) and α-Linolenic acid (ALA; 18:3n-3; $p=0.003$) concentrations were significantly higher in streams, however, DHA ($p=0.04$) in aquacultures. Iron (Fe), mercury (Hg) and selenium (Se) concentrations were significantly higher in stream ($p<0.05$). Results of stable isotope mixing models ($\delta^{13}C$, $\delta^{15}N$) indicated that aquaculture derived nutrients or heavy metals did not affect food webs of nearby streams.

Keywords: fatty Acids, heavy metals, aquatic food webs, salmonids

2.2. Introduction

Fish is as of high nutritional value for humans as it provides essential proteins and lipids. Recent research put particular emphasis on how lipids and their fatty acids are trophically conveyed to fish (Tidwell et al., 2007; Tocher et al., 2006) and eventually to humans (Cole et al., 2009; Domingo, 2009). Especially long chain polyunsaturated fatty acids (PUFA) of the omega-3 series are recognized as beneficial for brain and eye development in early stages of life (Santerre, 2010) and protective against cardiovascular diseases and mental disorders (Dewailly et al., 2007), which can be obtained through weekly consumption of oily fish, such as salmon.

Besides health benefit for humans, lipids also play a major role in organisms of the aquatic food web as they are constituents of cell membranes (structural lipids) and also provide repository energy (storage lipids). Several studies could demonstrate that the nutritional value of PUFA can enhance the somatic development of primary consumers of pelagic (Müller-Navarra et al., 2000) and fluvial food webs (Ghioni et al., 1996), as well as of fish (Berge et al., 2009; Nadezhda et al., 2007). Furthermore, lipid- and fatty acid composition of brood stock diet in aquacultures have been identified as major dietary factors that determine successful reproduction and survival of offspring (Izquierdo et al., 2000) and they have generally been recognized to be among the most important nutritional factors that affect fitness of aquatic organisms, supplying energy and essential compounds for general metabolic functioning, somatic growth, and reproduction (Müller-Navarra et al., 2000). Because these fatty acids or their precursors cannot be synthesized by animals on their own they are essential for humans and animals and are therefore called essential fatty acids (EFAs). In fact, the major primary producer taxa have distinctive fatty acid profiles, exclusively synthesized by themselves that may be transferred conservatively through the food web to consumers (Brett et al., 2006). Therefore, it is necessary to understand to what extent aquatic organisms retain PUFA along different trophic levels and at different aquatic habitats.

Crucial dietary omega-3 PUFA are eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), as well as the omega-6 PUFA arachidonic acid (ARA; 20:4n–6. While focusing research in the last years on EPA and DHA, the importance of ARA

has been largely neglected. Bell & Sargent (2003) depict a significant ARA requirement at specific stages in life cycle of fish and the necessity of this FA to cope with periods of environmental stress. Kainz et al. (2004) described that the most efficient transfer of ARA and EPA to fish in the consumption and assimilation of macrozooplankton, whereas predation on copepods results in the most efficient DHA uptake.

Beside lipid transfer in aquatic food webs there is evidence for bioaccumulation of heavy metals at different trophic levels (Kouba et al., 2010) that can be conveyed from the base to the top of the food chain. In addition to the natural occurrence of heavy metals in the environment, heavy metals also co-occur, at various levels, with essential nutrients of fish feeds used for aquaculture (Hites et al., 2004; Kelly et al., 2008). From an ecotoxicological point of view it is clear that aquatic food webs of natural aquatic systems versus aquaculture are exposed to different dietary routes of heavy metals. Moreover, it is clear that such different dietary trajectories may result in different bioavailability and -accumulation of heavy metals in organisms of natural aquatic systems versus aquaculture, and may eventually become potentially toxic to organisms. In aquatic ecosystems, heavy metals are ubiquitous and found at different concentrations in water, sediments, and aquatic organisms (Linnik and Zubenko, 2000). They can become serious threats to the food web because of their potential toxicity, long persistence, bioaccumulation and biomagnification. However, some concerns about potential health risks derived from contaminants in fish have been raised. Hence fish are a potential source of various environmental contaminants to humans. The accumulating extent of heavy metals in fish is by far dependent on the different metals, fish species, and their tissues, respectively (Chi et al., 2007). Therefore they all have different biomagnification potentials. Metals, such as cadmium (Cd), mercury (Hg), lead (Pb), selenium (Se) and zinc (Zn) may accumulate in the aquatic food chain and cause adverse effects on human health, whereas studies show that copper (Cu) is not biomagnified (Reinfelder et al., 1998). However, no common bioaccumulation potential of any trace metal or generally applicable bioaccumulation ratios exist. A lot of research in this field has been conducted, always showing different results on heavy metal BAFs of fish and their potential diets (i.e. Alibabic et al., 2007; Liang et al., 1999;). Pb and Cd are toxic elements that have no known biological function and may have carcinogenic effects on humans (Malik et al., 2010). Among all the heavy metals, mercury (Hg) has become the subject of most concern due to its

biomagnification potential and toxic effects to aquatic organisms and human health. The primary source of methyl mercury for humans is through the consumption of fish, and concentrations can reach very high level in predatory species (Holmes et al., 2009). So, if discussing desired health benefits of PUFA-containing farm-raised or wild fish, the risk of potential contamination should be considered concurrently.

However, the impact of heavy metals on macroinvertebrates has not been evaluated in terms of their food quality for fish of riverine ecosystems and adjacent aquaculture, even though invertebrates are a key food source for many lotic fish species (Iwasaki et al., 2009). All aquatic invertebrates accumulate trace metals in their tissues, either as metabolically available or detoxified metal (Rainbow, 2002). Many taxa are more or less sedentary and thus representative of local conditions and they are near the base of foodchains, so may be vital agents of metal entry into foodchains (Goodyear et al., 1999). Therefore they can be used as useful indicators for metal and nutrient entry through, i.e. water recharge from aquacultures to rivers. Accumulation of metals in fish tissues depends primarily on ambient water concentrations (bioconcentrations), metal concentrations of prey or commercial feed (biomagnification), such as chemical speciation/bioavailability as well as fish growth cycle, age, and trophic position (Kelly et al., 2008). Bioaccumulation factors (BAFs), the ratios of metal concentrations between consumers and diets, provide important information about differences in trophic transfer of metals.

To understand the coupling of lipid dynamics and heavy metal accumulation patterns in lotic environments it is necessary to investigate biota of different trophic levels. In this study we investigated fish farms expecting that their food chains consist of only two trophic levels (fish pellets and fish; i.e., controlled food chain) and natural riverine ecosystems, with phytobenthos at the base of their food chains, macrozoobenthos as intermediate consumers, and fish as aquatic top predators (natural riverine food chain).

To elucidate trophic positioning of lipids (physiologically required substances) as well as heavy metals (potential contaminants) in biota of aquaculture and fluvial ecosystems of pre-alpine Austria, we used stable nitrogen isotopes ($\delta^{15}N$). Minagawa & Wada (1984) and Post (2002) proposed an enrichment of 3.4 ± 1.1% of the $\delta^{15}N$ signature per trophic level due to respective fractionation processes. Signatures of $\delta^{13}C$ allow us to assess bulk carbon sources

and thus to determine which path an endconsumer's diet came from (Heissenberger et al., 2010).

Aquaculture fish are regarded to be highly beneficial for human health. In fact, depending on the salmon species and the diet fatty acid composition, farmed salmon can have PUFA contents in their flesh that are 2–5X higher than those in wild salmon (Ikonomou et al. 2007). However, aquaculture may also include negative effects on fish and those aquatic systems that are directly affect through the water outflow from farms. For example, contamination as well as increase of nutrients to streams (e.g., eutrophication) are known concerns of fish farms (Cole et al., 2009). Regarding the potential contamination of fish feed that is used in such farms we cannot obviate certain negative effects on nearby and eventually connected aquatic systems.

Encouraged by the ecotoxicological need to better understand how essential dietary nutrients (PUFA) and potentially toxic contaminants (heavy metals) are trophically transferred to fish in their natural habitats versus nearby fish farms, and how water discharge of fish farms may affect lipid and heavy metal concentrations in biota of streams partly recharged by aquaculture discharge, we designed a field study to examine; (i) trophic dependence of freshwater salmonids on benthic invertebrates and phytobenthos in pre-alpine riverine systems, (ii) the food-fish interaction in aquacultures and streams regarding PUFA retention patterns and heavy metal accumulation, and, (iii) possible effects of aquaculture on PUFA and heavy metal concentrations of natural riverine systems.

We tested the hypothesis that, (i) $\delta^{15}N$ signatures of consumers are enriched by ca. 3-4 ‰ relative to their diet and that the mean trophic fractionation of $\delta^{13}C$ is <1 ‰ (Post 2002), (ii) fish from aquacultures have both, higher concentrations of heavy metals and PUFA compared to fish from natural habitats, and, (iii) benthic organisms of the streams receiving water from aquaculture show higher heavy metal and PUFA concentrations than at the sampling sites before the water inflow to aquacultures. Therefore, it is assumed that there is an effect of aquacultures on stream organisms and that their essential fatty acid and heavy metal profiles generally differ between the two scrutinized aquatic systems, aquacultures and streams.

2.3. Materials and Methods

The study area was located in pre-alpine Lower Austria in the region of the Northern Limestone Alps. Four fish farms (AC1-4) and four streams (Bodingbach *B*, Lassingbach *L*, Mendlingbach *M* and Rohrer-Zellenbach *Z*) up- and downstream of the respective aquacultures were sampled to investigate retention of total lipids and their omega-3 and -6 PUFA, MUFA, SAFA, and heavy metals (Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn) in macrozoobenthos (fish food of streams), pellet feeds (fish farms), and fish (streams and fish farms) (Fig. 1). Sampling was carried out during September and October 2009. Samples of riverine fish were taken within the trout region (low temperatures, highly oxygenated, high current, and gravel/rock underground) of these streams. Abiotic parameters (temperature, conductivity, oxygen saturation, pH and flow rate) have been collected in all four streams (Table 1).

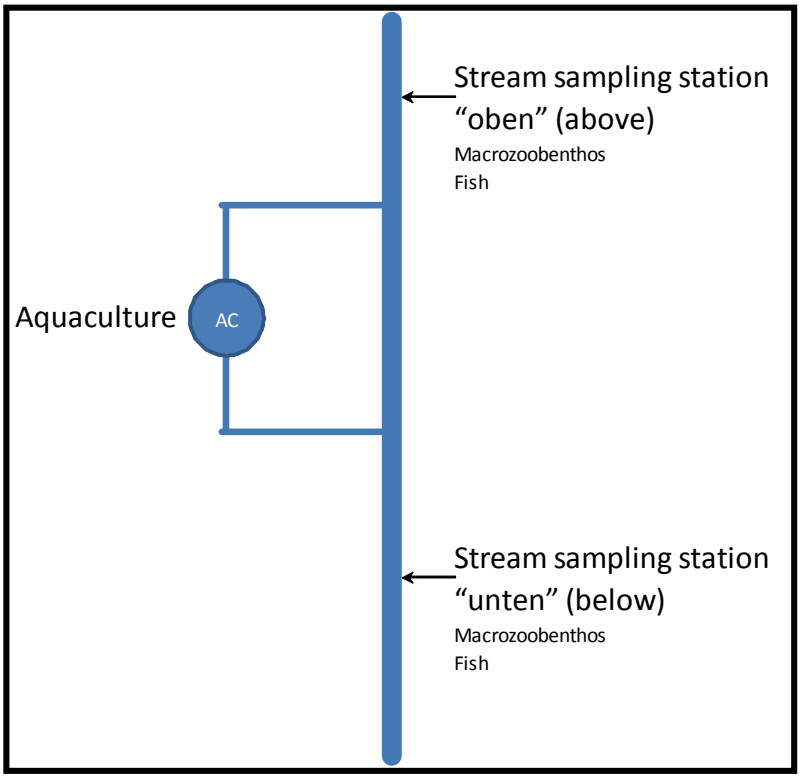

Figure 1 Schematic graph of the four streams (B, L, M and Z). At each of them we had three sampling stations: above (upstream), aquaculture (AC) and below (downstream).

Fish farms were fed by water from these streams and subsequently discharged back to these streams. Hence, we sampled aquatic biota, including fish and macrozoobenthos in these streams before and after the fish farms, and fish feeds and fish were sampled in the fish farms.

Brook trout (*Salmo trutta fario*), rainbow trout (*Oncorhynchus mykiss*), and Arctic charr (*Salvelinus alpinus lepeschini;* used in Austrian aquaculture*)* were caught by hand nets (fish farms) and electrofishing (streams) (n=3 per sampling site), and their body size and weight were immediately measured.

Macrozoobenthos (n=2 per sampling station) were collected randomly by hand and their taxa identified. All samples were shock frozen (-80˚C to limit possible lipolytic degradation), freeze-dried, homogenized, and stored at -80°C until further analysis.

2.3.1. Lipid analysis

Lipid analysis was performed on pure white dorsal fish muscle samples, two types of fish food, natural food (benthic invertebrates) and artificial pellets (Danex^{TM}) and phytobenthos. Details of lipid and fatty acid analyses are described elsewhere (Heissenberger et al. 2010). In brief, chloroform (2 mL) was added to the freeze-dried, homogenized samples and stored overnight under N_2 atmosphere at -80°C. After lipid extraction the amount of total lipids was determined gravimetrically by weighing aliquots of lipid extracts on a microscale (±1 μg; Gibertini TM) as a measure for the required quantity for subsequent formation of fatty acid methyl esters (FAME), which were analyzed using a gas chromatograph (TRACE GC THERMO, Detector: FID 260°C, Carrier gas: H2:40 ml/min, N2: 45 ml/min, air: 450 ml/min, temperature ramp: 140°C (5 min)–4°C/min–240°C (20 min) = 50 min) equipped with a temperature-programmable injector and an autosampler.

2.3.2. Heavy metal analysis

All samples (fish, commercial and natural feed) were analyzed for heavy metals (Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn). For mineralization, aliquots (100 – 300 mg) of the samples were weighted to 0.1 mg in quartz digestion vessels. After addition of nitric acid (3 mL) and hydrogenperoxide (0.5 mL; p.a., 30%) to the samples, certified reference material (DORM-2

Dogfish *Muscle Certified Reference Material for Trace Metals*) and digestion blanks were digested in Anton Paar Multiwave 3000. After cooling the digests were quantitatively transferred to 50 ml polyethylene tubes and diluted to a final volume of 30 ml with MilliQ water (> 18.2 MΩ*cm).

The determination of mercury (Hg) was performed with flow injection cold vapor generation atomic absorption spectrometry (Perkin Elmer FIMS 400) according to ÖNORM EN 1483 (modified). Quantitative determination was performed with matrix matched aqueous standard solutions. For quality control standard reference material NIST 1641d was used.

The determination of other heavy metals (*Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se Sn,* and *Zn*) was performed with inductively coupled plasma mass spectrometry (Perkin-Elmer ELAN DRC II) according to ÖNORM EN ISO 17294-2 (modified). Quantitative determination was performed with matrix matched mixed aqueous standard solutions. For quality control standard reference material NIST 1643e was used.

2.3.3. Stable isotope analyses, ^{13}C lipid correction and estimating dietary contribution

Freeze-dried samples of fish (dorsal muscle tissues), fish pellets, benthic invertebrates, and phytobenthos were homogenized and weighed. Analyses of δ^{15}N and δ^{13}C were conducted using an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) interfaced via a ConFlo II device (Finnigan MAT, Bremen, Germany) to a continuous flow stable isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany). The reference gas N_2 (Air Liquide) was calibrated to the at-air international standard using IAEA-N-1, IAEA-N-2 and IAEA-NO-3 (IAEA, Vienna, Austria) and reference gas CO_2 to IAEA-CH-6 and IAEA-CH-7 reference material (both International Atomic Energy Agency, Vienna, Austria).

All stable isotope values were reported in the □ notation where δ^{13}C or δ^{15}N = ([Rsample/ Rstandard] - 1) 9 1000, where R is ^{13}C:^{12}C or ^{15}N:^{14}N. We used 0.15% for δ^{15}N and 0.10% for δ^{13}C standard deviation of measurement.

A lipid correction for δ^{13}C values based on the model of McConnaughey and McRoy (1979) was applied, as values for δ^{13}C in lipids are depleted compared to values of lipid extracted tissues (Kiljunen *et al.* 2006). The model uses the C:N ratio as a proxy for total lipid contents

and normalizes $\delta^{13}C$ values of samples varying to large extent in lipid concentrations. Formulas were defined as:

$$L = \frac{93}{1+(0.246\times(C:N)-0.775)^{-1}} \qquad \textbf{eq. 1}$$

$$\delta^{13}C' = \delta^{13}C + D\times(I+\frac{3.90}{1+287/L}) \qquad \textbf{eq. 2}$$

where $\delta^{13}C'$ is the lipid-normalized value of the sample and $\delta^{13}C$ is the measured value of the sample. *L* represents the proportional lipid content of the sample and C:N is the proportions of carbon and nitrogen in the sample. The protein-lipid discrimination (*D*) was set to 7‰ as suggested by Sweeting et al. (2007). *I* is a constant and assigned a value of −0.207.

The dietary contribution of added feeds to the macrozoobenthos and fish in streams and salmonids in fish farms was assessed using SIA*R* (Stable Isotope Analysis in *R*). Fractionation factors of ^{13}C and ^{15}N between trophic levels were set at 1.1 ± 0.3‰ and 2.8 ± 0.4‰ for C and N, respectively (McCutchan et al., 2003).

Phytobenthos, benthic invertebrates and the additional fish feeds were used as the only potential food sources in the mixing models.

2.3.4. Statistical analysis

Data of fatty acid and heavy metal concentrations were log-transformed to ensure normal-distribution for parametric tests. To compare fatty acid and heavy metal concentrations of streams and aquacultures t-test analysis was used.

Relationships between total lipid proportions and individual metal concentrations of salmonids were tested using linear regression models (Spearson´s correlation coefficient; R^2).

Differences between means of parameters upstream, aquaculture, and downstream were analyzed using analysis of variance (ANOVA) followed by Tukey *post hoc* test.

The level of significance of all statistical tests was set to $p<0.05$. Statistical analyses were performed using SPSS (17.0) and *R* (2.12.2).

2.3. Results

Water temperatures ranged between 11.6°C (Zellenbach down) and 9°C (Lassingbach down), oxygen saturation between 10.44 mgl^{-1} (Bodingbach up) and 12.30 mgl^{-1} (Zellenbach up) and conductivity between 388 µS cm^{-1} (Zellenbach up) and 280 µS cm^{-1} (Bodingbach down). The pH values were alkaline and nearly identical in all four streams throughout the study ranging from 8.2 to 8.4 (Table 1). River benthos consisted of insect larvae of the order Trichoptera, Ephemeroptera, Plecoptera, Diptera, Crustaceae (Gammaridae) and Oligochaeta, all aquacultures provided pigmented fish feed (pellets) (Table 2).

Table 1 Geographic coordinates and abiotic parameters of study streams (Bodingbach, B; Mendlingbach, M; Zellenbach, Z; Lassingbach, L; u, upstream, d, downstream)

	Bu	Bd	Mu	Md	Zu	Zd	Lu	Ld
longitude	O 15° 00,722'	O 15° 01,008'	O 14° 52,018'	O 14° 52,122'	O 15° 45,775'	O 15° 45,206'	O 14° 54,338'	O 14° 54,441'
latitude	N 47° 52,068'	N 47° 51,898'	N 47° 53,054'	N 47° 45,245'	N 47° 52,776'	N 47° 53,054'	N 47° 44,981'	N 47° 45,493'
temperature [°C]	10.8	9.6	10.8	11.2	11.3	11.6	9.6	9.0
O2 saturation [%]	118	104	104.9	104.2	101.6	104	102.2	106.7
O2 saturation [mgl^{-1}]	12.3	11.2	11.01	10.84	10.44	10.64	10.98	11.63
pH	8.4	8.3	8.3	8.4	8.2	8.2	8.4	8.2
conductivity [μScm^{-1}]	289	280	351	350	388	387	293	363
mean flow rate [$m3s^{-1}$]	0.65	0.65	0.65	0.95	0.6	0.6	0.7	0.65
mean depth [m]	0.4	0.3	0.15	0.3	0.25	0.2	0.15	0.2

Table 2 Potential diet composition of the streams (Bodingbach, B; Mendlingbach, M; Zellenbach, Z; Lassingbach, L; u, upstream; d, downstream) and the aquacultures (AC 1-4)

Aquatic system	Analyzed potential diet
Bu	Dayflies (Ephemeridae, Heptageniidae, Baetidae), Stoneflies (Perlidae), Dipterans (Simuliidae), Caddiesflies (Hydropsychidae)
Bd	Crustacean (Gammaridae), Dayflies (Baetidae), Stoneflies (Perlidae), Dipterans (Simuliidae), Caddiesflies (Hydropsychidae)
Lu	Crustacean (Gammaridae), Dayflies (Baetidae), Stoneflies (Perlidae), Dipterans (Simuliidae), Caddiesflies (Hydropsychidae)
Ld	Crustacean (Gammaridae), Dayflies (Baetidae), Stoneflies (Perlidae), Dipterans (Simuliidae), Caddiesflies (Rhyacophilidae)
Mu	Dayflies (Baetidae), Stoneflies (Heptageniidae, Perlidae), Dipterans (Simuliidae), Caddiesflies (Hydropsychidae, Rhyacophilidae)
Md	Dayflies (Baetidae), Stoneflies (Perlidae), Coleopterans (Elmidae), Dipterans (Simuliidae), Caddiesflies (Philopotamidae)
Zu	Crustacean (Gammaridae), Stoneflies (Nemouridae Perlidae), Dipterans (Athericidae), Caddiesflies (Hydropsychidae)
Zd	Crustacean (Gammaridae), Dayflies (Baetidae), Stoneflies (Perlodidae), Dipterans (Simuliidae), Caddiesflies (Hydropsychidae)
AC1	Pellets, DanexTM
AC2	Pellets, DanexTM
AC3	Pellets-AlpenlachsTM
AC4	Pellets-AlpenlachsTM

2.4.1. Total lipids and Fatty Acids

Total lipid concentrations of salmonids did not differ significantly between the two aquatic habitats (p=0.5): aquacultures (110.6 ± 35.4 mg/g) and streams (120.5 ± 49.6 mg/g), whereas among the potential diets (p=0.007): total lipid concentrations of pellets (242.1 ± 58.2 mg/g) were significantly higher than in macrozoobenthos (177.2 ± 47.0 mg/g). There was no significant difference (p=0.3) between total PUFA concentrations of pellets (4.3 ± 5.5 mg/g dw) and riverine benthos (5.2 ± 6.0 mg/g dw), or among aquacultures (29.9 ± 8.9 mg/g dw) and streams (29.7 ± 9.1 mg/g dw; p=0.8). Moreover, total lipid concentrations were not significantly different between fish from stream and aquaculture (p=0.5). Concentrations of total PUFA, MUFA, SAFA and individual EFAs of fish and potential diets at the four sampling sites are listed in Table 3 and 4.

Comparing individual EFA concentrations of fish between streams and aquacultures, no significant difference of Linoleic acid (LIN; 18:2n-6; p= 0.6) and EPA (p=1.0) concentrations were.

Arachidonic acid concentrations were significantly higher (p<0.001) of riverine salmonids (1.5 ± 0.1 mg/g dw) than of fish farms (0.6 ± 0.0 mg/g dw). Similarly, alpha-linolenic acid (ALA; 18:3n-3) concentrations were significantly higher (p = 0.003) in streams (3.0 ± 0.4 mg/g dw) than in AC (1.2 ± 0.2 mg/g dw). However, DHA concentrations were significantly lower (p=0.04) in fish from streams (13.1 ± 0.8 mg/g dw) than farms (15.9 ± 1.0 mg/g dw).

The same analysis was done for potential diets (macrozoobenthos and pellets) in streams and aquacultures. LIN (p<0.001) and DHA (p<0.001) concentrations were significantly higher in aquaculture feeds (14.1 ± 4.9 and 9.8 ± 3.8 mg/g dw) than in potential riverine diets (7.3 ± 1.4 and 0.3 ± 0.2 mg/g dw). ARA (p=0.01), ALA (p<0.001) and EPA (p=0.01) concentrations of potential diets were significantly higher in streams (1.7 ± 0.4, 9.2 ± 5.3 and 14.2 ± 3.0 mg/g dw) than in fish farms (0.7 ± 0.2, 3.3 ± 1.7 and 10.7 ± 3.2 mg/g dw).

We calculated the PUFA retention ratios between fish and potential diets for every single habitat by dividing PUFA concentrations of fish by PUFA concentrations of their potential diet (i.e., $[PUFA]_{fish}/[PUFA]_{diet}$) to assess the trophic relationship between dietary PUFA supply and PUFA retention in the consumer. The higher these ratios, the more the PUFA

compounds were retained (Fig. 2). Bioaccumulation of DHA was strongest in fish of all streams and aquacultures and accumulated distinctly more in lotic environments, ARA was accumulated in AC 2-4 and LIN showed bioaccumulation in Bu.

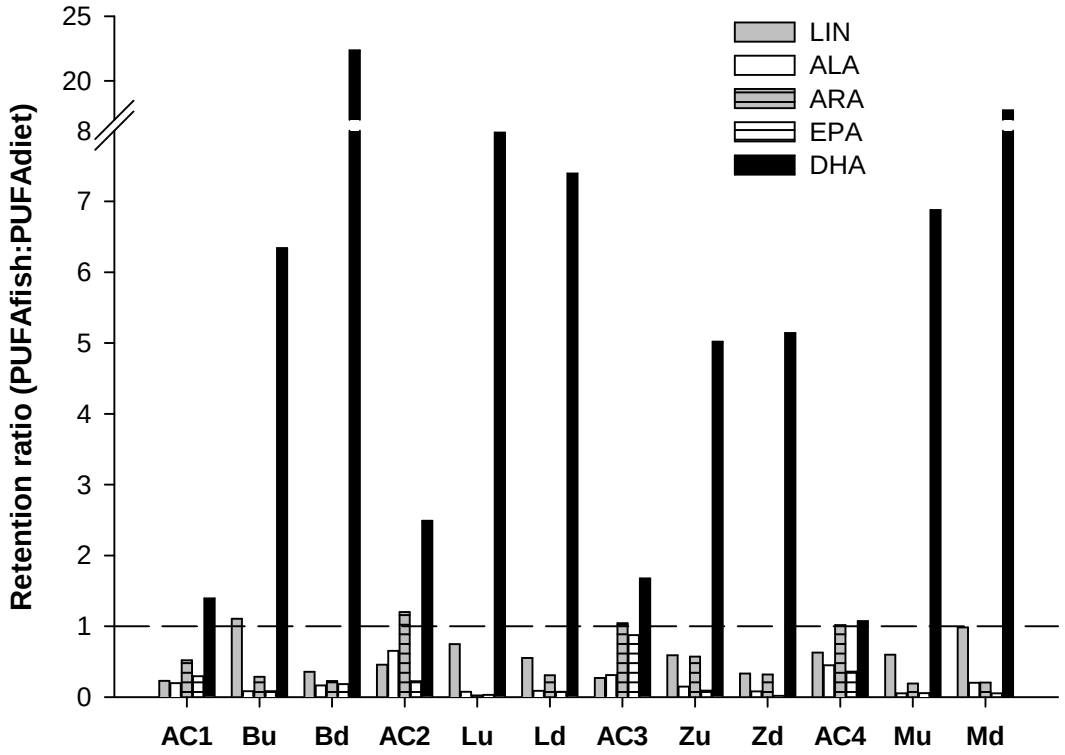

Figure 2 Retention ratios of polyunsaturated fatty acids between fish and potential diets in streams (concentration of PUFAfish/PUFAdiet). (*B*, Bodingbach; *L*, Lassingbach; *M*, Mendlingbach; *Z*, Zellenbach; *u*, up; *d*, down; *AC1-4*, Aquaculture 1-4; LIN linoleic acid, ALA a-linolenic acid, ARA arachidonic acid, EPA eicosapentaenoic acid, DHA docosahexaenoic acid)

Table 3 Fatty acids concentrations (PUFA; mg/g dw) of riverine and aquaculture-raised salmonids

	18:2*n* - 6	18:3*n* - 3	20:4*n*-6	20:5*n* - 3	22:6*n* - 3	SAFA	MUFA	n-3 PUFA	n-6 PUFA	Total PUFA	Total Lipids
ST-Bu	8.5 ± 7.4	1.7 ± 1.1	1.4 ± 0.5	3.1 ± 1.4	8.5 ± 1.7	19.1 ± 14.4	26.2 ± 10.3	13.3 ± 4.2	13.0 ± 10.1	26.3 ± 3.8	91.6 ± 51.1
ST-Bd	2.3 ± 2.6	1.9 ± 1.9	1.0 ± 0.4	5.1 ± 2.9	18.4 ± 6.6	12.0 ± 2.8	9.0 ± 2.6	20.1 ± 11.5	6.3 ± 6.5	26.3 ± 3.3	109.7 ± 45.5
ST-Lu	4.8 ± 1.3	5.3 ± 1.5	2.0 ± 0.0	3.9 ± 0.4	11.1 ± 1.5	15.6 ± 3.8	15.9 ± 5.5	20.6 ± 1.7	10.1 ± 1.9	30.7 ± 0.7	96.2 ± 12.2
ST-Ld	4.2 ± 3.0	3.4 ± 1.2	1.5 ± 0.5	3.6 ± 1.2	13.5 ± 4.1	13.7 ± 3.3	12.7 ± 4.3	20.8 ± 3.4	8.7 ± 2.6	29.5 ± 0.3	82.8 ± 8.7
ST-Zu	5.2 ± 6.0	2.5 ± 1.5	2.1 ± 1.0	3.7 ± 1.0	13.2 ± 1.1	15.5 ± 3.7	16.9 ± 6.5	19.6 ± 3.5	10.4 ± 9.5	29.9 ± 0.3	150.7 ± 54.0
ST-Zd	2.9 ± 1.8	2.1 ± 0.9	1.9 ± 0.7	4.0 ± 0.2	14.8 ± 3.2	11.3 ± 2.8	8.7 ± 3.0	21.1 ± 3.0	7.1 ± 2.5	28.1 ± 0.9	211.3 ± 26.5
ST-Mu	3.8 ± 0.3	2.5 ± 0.7	1.0 ± 0.2	4.5 ± 0.8	13.4 ± 2.2	14.0 ± 3.3	13.6 ± 4.3	20.6 ± 0.8	8.4 ± 0.6	29.0 ± 3.4	103.1 ± 24.6
ST-Md	6.0 ± 4.2	5.2 ± 2.6	1.2 ± 0.3	5.2 ± 0.7	16.8 ± 3.8	20.1 ± 4.5	19.3 ± 5.8	27.5 ± 5.1	12.7 ± 5.7	40.2 ± 0.5	115.3 ± 41.0
ST-AC1	3.5 ± 1.0	1.0 ± 0.3	0.5 ± 0.1	3.2 ± 0.3	16.9 ± 1.6	13.6 ± 3.1	12.3 ± 3.7	21.1 ± 2.2	6.2 ± 1.6	27.3 ± 2.8	79.0 ± 22.0
OM-AC2	3.6 ± 1.4	0.6 ± 0.2	0.5 ± 0.0	3.2 ± 0.7	12.5 ± 0.3	9.9 ± 2.4	8.5 ± 2.8	16.3 ± 0.6	5.6 ± 2.0	21.9 ± 0.9	111.6 ± 14.8
SL-AC3	5.1 ± 3.5	1.3 ± 0.9	0.7 ± 0.2	6.1 ± 2.7	17.3 ± 5.0	17.3 ± 4.1	20.5 ± 6.0	24.8 ± 8.6	9.1 ± 6.0	33.9 ± 0.6	106.9 ± 50.2
OM-AC4	9.3 ± 1.5	1.8 ± 0.2	0.6 ± 0.0	3.7 ± 0.3	17.1 ± 0.5	20.3 ± 4.8	23.4 ± 6.4	22.8 ± 0.6	13.8 ± 2.1	36.6 ± 0.5	144.8 ± 20.2

B *Bodingbach,* L *Lassingbach,* Z *Zellenbach,* M *Mendlingbach,* AC *Aquaculture,* ST Salmo trutta fario , OM *Oncorhynchus mykiss*, SL Salvelinus *lepeschini,* u *upstream*, d *downstream*;

Table 4 Fatty acids concentrations (PUFA; mg/g dw) of potential salmonid diet from different habitats

	18:2*n* - 6	18:3*n* - 3	20:4*n*-6	20:5*n* - 3	22:6*n* - 3	SAFA	MUFA	n-3 PUFA	n-6 PUFA	Total PUFA	Total Lipids
mzb-Bu	7.7 ± 2.3	13.8 ± 3.0	1.9 ± 0.5	17.4 ± 4.5	0.3 ± 0.1	38.6 ± 22.9	29.9 ± 8.9	31.6 ± 7.7	24.1 ± 12.8	55.6 ± 20.4	206.2 ± 94.5
mzb-Bd	6.4 ± 1.1	11.8 ± 1.4	2.0 ± 0.6	15.7 ± 0.3	0.3 ± 0.1	30.5 ± 4.2	29.1 ± 9.8	27.8 ± 1.9	16.6 ± 1.8	44.5 ± 3.6	180.8 ± 12.1
mzb-Lu	6.4 ± 1.0	15.6 ± 2.1	1.8 ± 0.0	12.8 ± 0.1	0.2 ± 0.1	28.0 ± 5.3	21.6 ± 4.5	28.8 ± 2.1	15.8 ± 6.5	44.5 ± 8.2	138.1 ± 16.0
mzb-Ld	7.6 ± 1.8	13.9 ± 1.4	1.7 ± 0.2	15.6 ± 5.5	0.5 ± 0.5	41.9 ± 5.8	31.3 ± 3.6	30.2 ± 10.8	19.7 ± 9.0	49.9 ± 19.7	258.2 ± 26.4
mzb-Zu	8.9 ± 1.0	10.2 ± 4.3	1.7 ± 1.0	11.4 ± 1.6	0.2 ± 0.3	26.8 ± 15.7	20.1 ± 7.5	21.9 ± 6.4	15.8 ± 5.2	37.7 ± 11.5	172.7 ± 15.0
mzb-Zd	8.7 ± 0.1	11.2 ± 1.8	2.2 ± 0.3	12.5 ± 1.8	0.6 ± 0.4	27.1 ± 4.5	26.7 ± 4.4	24.4 ± 3.1	15.3 ± 0.9	39.7 ± 4.1	152.4 ± 24.5
mzb-Mu	6.3 ± 0.1	12.6 ± 2.0	1.3 ± 0.3	13.9 ± 0.7	0.3 ± 0.1	28.8 ± 9.4	23.1 ± 1.3	27.0 ± 2.6	19.2 ± 6.0	42.2 ± 8.5	166.7 ± 21.1
mzb-Md	6.1 ± 1.5	13.1 ± 6.2	1.5 ± 0.0	14.0 ± 2.5	0.2 ± 0.0	30.4 ± 17.2	21.0 ± 4.6	27.4 ± 8.7	17.7 ± 8.9	45.1 ± 17.6	142.3 ± 4.5
AC-1	15.4 ± 4.5	4.7 ± 1.4	0.9 ± 0.2	11.0 ± 3.0	12.1 ± 3.0	34.7 ± 10.1	49.2 ± 13.8	28.1 ± 7.5	22.7 ± 6.4	50.8 ± 14.0	225.3 ± 50.0
AC-2	7.8 ± 0.8	0.9 ± 0.0	0.5 ± 0.0	14.2 ± 1.8	5.0 ± 0.7	33.1 ± 0.8	21.4 ± 1.7	20.1 ± 2.6	12.8 ± 1.3	32.9 ± 3.9	171.1 ± 20.6
AC-3	18.9 ± 1.3	4.1 ± 0.3	0.7 ± 0.0	7.0 ± 0.6	10.3 ± 0.9	34.0 ± 2.9	38.7 ± 3.3	21.6 ± 1.8	25.1 ± 1.8	46.7 ± 3.6	274.1 ± 18.6
AC-4	14.8 ± 1.2	4.1 ± 0.3	0.6 ± 0.1	10.3 ± 1.0	15.9 ± 1.4	54.4 ± 7.2	49.8 ± 4.1	30.6 ± 2.7	26.5 ± 2.2	48.7 ± 7.0	298.0 ± 39.1

B *Bodingbach,* L *Lassingbach,* Z *Zellenbach,* M *Mendlingbach,* AC *Aquaculture,* mzb *riverine macrozoobenthos*, u *upstream*, d *downstream*

2.4.2. Heavy Metals

In muscle samples of salmonids from aquacultures the average concentration of heavy metals, namely Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn were 0.001 ± 0.01, 0.2 ± 0.3, 1.8 ± 1.5, 11.0 ± 4.4, 0.1 ± 0.0, 0.5 ± 0.1, 0.04 ± 0.04, 0.02 ± 0.03, 0.7 ± 0.09, 0.2 ± 0.2 and 18.2 ± 2.3 mg kg^{-1}, respectively. Fish from streams had average heavy metal concentrations, mentioned in the same order as above: 0.01 ± 0.01, 0.2 ± 0.2, 1.7 ± 0.3, 24.6 ± 13.7, 0.2 ± 0.1, 0.5 ± 0.1, 0.03 ± 0.07, 0.01 ± 0.01, 1.1 ± 0.3, 0.2 ± 0.6 and 17.0 ± 2.7 mg kg^{-1} (Table 5 and 6).

In general, comparing individual heavy metal concentrations of fish between streams and aquacultures no significant difference was found for Cd (p=0.1), Cr (p=0.7), Cu (p=0.7), Mn (p=0.7), Ni (p=0.7), Pb (p=0.3), Sn (p=0.7) and Zn (p=0.2). However, Fe concentrations of fish in streams (24.6 ± 13.7 mg kg^{-1}) were significantly higher (p = 0.002) than in aquacultures (11.1 ± 4.4 mg kg^{-1}). Also, Hg concentrations of fish in streams (0.2 ± 0.1 mg kg^{-1}) were significantly higher (p=0.002) than in aquacultures (0.1 ± 0.04 mg kg^{-1}) and Se concentrations (p<0.001) were also higher in riverine habitats (1.1 ± 0.3 mg kg^{-1}) than in aquacultures (0.7 ± 0.1 mg kg^{-1}).

The same analysis was done for potential diets (macrozoobenthos and pellets) in streams and aquacultures. No significant differences could be found for Cu (p=0.12), Hg (p=0.11), Mn (p=0.09), Sn (p=0.09) and Zn (p=0.4). Cd (p=0.01), Cr (p=0.03), Fe (p=0.04), Ni (p=0.03), Pb (p=0.0002) and Se (p=0.001) concentrations were significantly higher in potential riverine diets (1.36 ± 1.17, 4.40 ± 3.88, 899.38 ± 676.34, 3.06 ± 1.79, 2.23 ± 1.33 and 3.87 ± 2.05 mg kg^{-1}) than in aquaculture feeds (0.24 ± 0.12, 1.21 ± 0.55,358.75 ± 58.66, 1.58 ± 0.13, 0.14 ± 0.06 and 1.22 ± 0.59 mg kg^{-1}).

Heavy metal concentrations of benthic invertebrates were all not significantly different between up- and downstream sampling sites (p>0.05).

Increasing total lipid concentrations of dorsal muscle tissue predicted 41% of the variability of Hg concentrations in these salmonids (p=0.02, R^2=0.41; Fig.3).

No significant relationship between total lipid and all other metal concentrations (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, Zn) were found in fish of streams or aquaculture.

Linear regression analysis was also conducted to test relationships between metal concentrations of aquaculture diet and raised fish. Fish feeds predicted Mn and Se concentrations of fish tissue (p=0.03, $R^{2=}$0.80; p=0.004, R^2=0.90, respectively). Heavy metal

concentrations of macrozoobenthos only had predictive power (60%) for Se concentration in riverine fish (p=0.03, $R^{2=}$0.60).

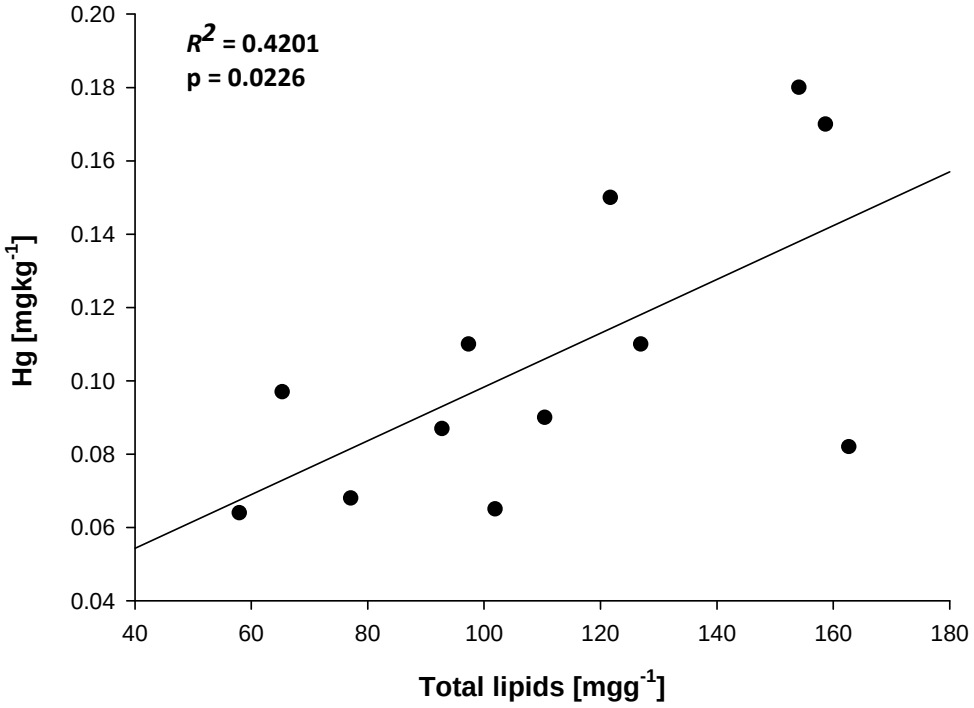

Figure 3 Results of linear regression analysis conveyed increasing Hg concentrations of salmonids in aquacultures when total lipid concentration was increasing (p=0.02, R^2=0.41).

Furthermore, to draw the scientific focus on the individual habitats, heavy metal retention ratios were calculated between fish and potential diets (i.e., pellets and macrozoobenthos for aquaculture and riverine fish, respectively), by dividing heavy metal concentrations of fish by heavy metal concentrations of their potential diet (i.e., [heavy metal]fish/[heavy metal]diet) to assess the trophic relationship between dietary metal supply and metal retention in the consumer. Based on these retention ratio values it was demonstrated (Fig 3.a/b) that the only two metals showing bioaccumulation in fish from streams and aquacultures were mercury (Hg) and tin (Sn; Fig. 4).

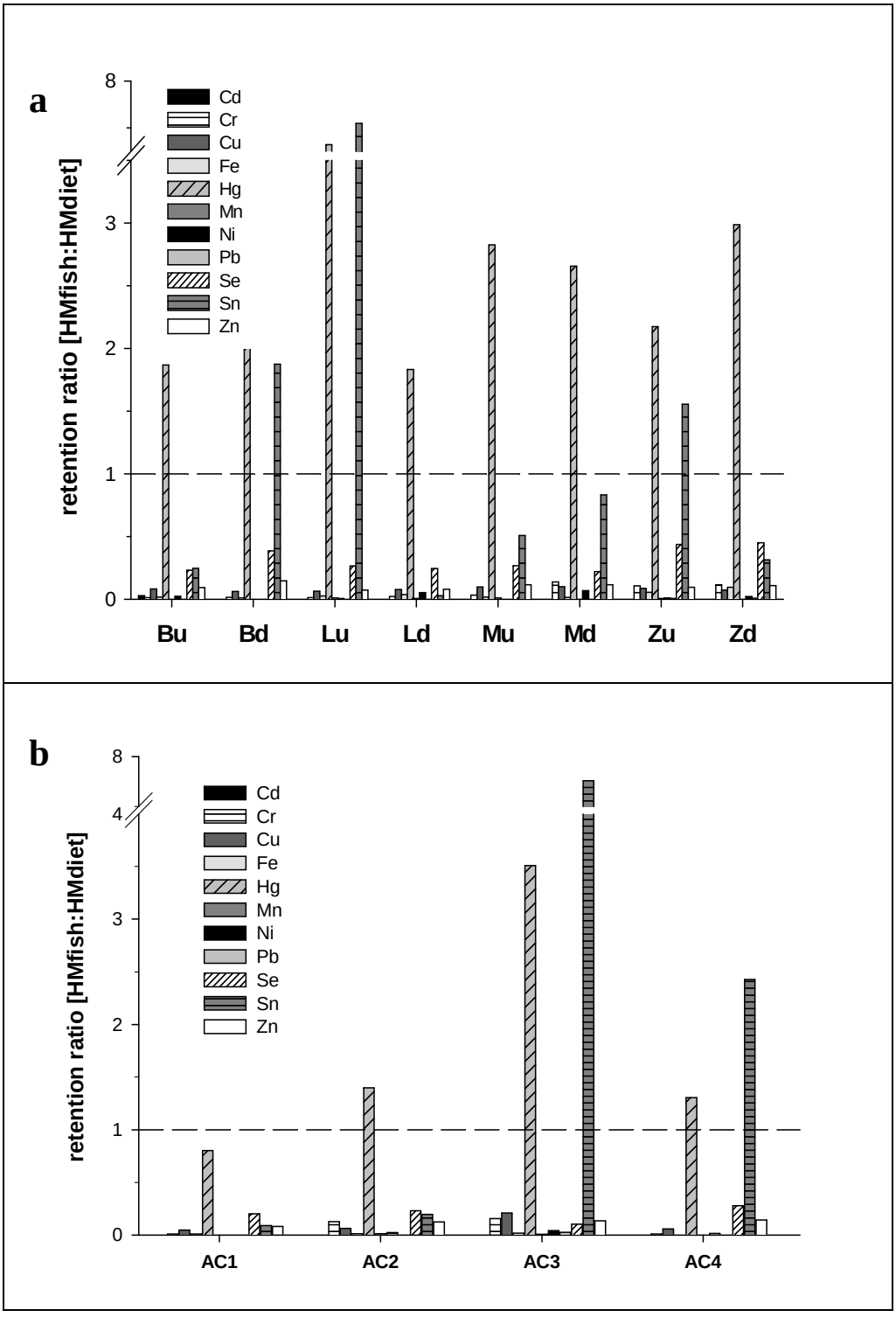

Figure 4 a/b: Retention ratios of heavy metals between fish and potential diets in streams (concentration of HMfish/HMdiet). (*B*, Bodingbach; *L*, Lassingbach; *M*, Mendlingbach; *Z*, Zellenbach; *u*, up; *d*, down)

Table 5 Heavy metal concentrations (mg kg-1) of potential diets: pellets in aquacultures (AC1-4) and macrozoobenthos of the 4 streams (B, Bodingbach; L, Lassingbach; M, Mendlingbach; Z, Zellenbach; u, upstream of aquacultures; d, downstream of aquacultures).

	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
AC1	0.84	9.85	25.00	680.00	0.08	46.50	5.15	3.80	3.30	0.37	185.00
AC2	0.87	3.85	23.00	1350.00	0.07	47.50	2.80	1.70	2.80	0.11	160.00
AC3	2.95	1.47	15.00	695.00	0.05	87.00	2.30	1.60	7.35	0.04	135.00
AC4	0.62	7.80	26.00	1745.00	0.07	179.50	5.00	2.30	2.85	0.13	135.00
Bu	0.77	4.75	20.00	1000.00	0.06	88.50	3.25	1.77	3.80	0.11	150.00
Bd	0.62	7.80	26.00	1745.00	0.07	179.50	5.00	2.30	2.85	0.13	135.00
Lu	2.27	8.95	24.00	825.00	0.06	39.00	4.50	2.15	5.50	0.08	210.00
Ld	2.08	3.90	20.50	500.00	0.09	67.50	3.15	4.80	4.45	0.54	205.00
Mu	2.00	3.10	19.00	1105.00	0.06	41.50	2.65	1.45	4.60	0.10	150.00
Md	1.82	2.22	19.00	940.00	0.06	93.00	2.45	1.85	5.55	0.06	145.00
Zu	0.63	1.80	16.00	595.00	0.09	65.00	1.23	1.49	1.85	0.63	170.00
Zd	0.72	2.70	23.50	485.00	0.10	210.00	2.25	2.05	2.35	0.06	175.00

Table 6 Heavy metal concentrations (mg kg-1) of fish: aquacultures (AC1-4) and streams (B, Bodingbach; L, Lassingbach; M, Mendlingbach; Z, Zellenbach; u, upstream of aquacultures; d, downstream of aquacultures).

	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
AC1	0.00	0.08	1.13	5.73	0.07	0.27	0.03	0.00	0.67	0.03	15.00
AC2	0.00	0.49	1.44	16.33	0.10	0.59	0.06	0.01	0.64	0.02	20.00
AC3	0.00	0.23	3.13	11.90	0.17	0.58	0.10	0.04	0.75	0.33	18.33
AC4	0.00	0.07	1.50	10.33	0.09	0.44	0.07	0.01	0.79	0.30	19.33
Bu	0.02	0.07	1.63	17.67	0.11	0.43	0.08	0.01	0.88	0.03	14.00
Bd	0.00	0.13	1.67	21.33	0.21	0.39	0.00	0.00	1.10	0.23	20.00
Lu	0.01	0.13	1.57	22.33	0.31	0.46	0.03	0.01	1.47	0.52	15.33
Ld	0.01	0.09	1.60	18.67	0.16	0.46	0.17	0.01	1.10	0.02	16.33
Mu	0.00	0.10	1.87	21.00	0.18	0.46	0.00	0.00	1.23	0.05	17.67
Md	0.00	0.31	1.90	16.33	0.16	0.45	0.17	0.00	1.23	0.05	17.00
Zu	0.00	0.19	1.40	32.67	0.20	0.48	0.01	0.01	0.81	0.98	16.33
Zd	0.00	0.32	1.70	46.67	0.29	0.54	0.05	0.02	1.06	0.02	19.00

2.4.3. Food Web Analysis, Mixing Models and potential effect of aquacultures on streams

Stable nitrogen values ($\delta^{15}N$) were highest in aquaculture fish (mean 10.75 ± 0.67‰). In contrast, the $\delta^{15}N$ values of their diet (pellets) were consistently lower, 6.46 ± 2.49‰. Fish of the 4 investigated streams had on average a $\delta^{15}N$ signature of 6.13 ± 1.02‰, benthic invertebrates 1.57 ± 0.81‰, and phytobenthos -0.82 ± 1.51‰. The corresponding $\delta^{13}C$ values were: fish aquacultures (-21.98 ± 0.17‰), diet aquacultures (-21.43 ± 0.53‰), fish streams (-29.12 ± 0.40‰), benthic invertebrates (-32.52 ± 1.69‰) and algae (-26.04 ± 5.11‰; Fig. 5).

The average isotopic difference of $\delta^{15}N$ between diet and consumer in fish farms was 4.3‰ and the mean fractionation of $\delta^{13}C$ between diet and fish 0.55 ‰. However, $\delta^{15}N$ and $\delta^{13}C$

fractionation between diet and fish of streams was different: between benthic invertebrates and fish 4.56‰ and 3.4‰, among phytobenthos and invertebrates 2.38‰ and 6.48‰, respectively (Table 7).

Table 7 Isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) of macrozoobenthos and fish in streams and aquacultures supplemented by its fractionation factors. These were calculated dividing values of the consumer by its potential diet (expecting that macrozoobenthos feeds on phytobenthos, fish from streams on macrozoobenthos and fish from aquacultures on fish pellets).

	Isotopic ratios		Fractionation factor	
	$\delta^{15}N$	$\delta^{13}C$	$\Delta^{15}N$	$\Delta^{13}C$
MZB-B	1.86	-34.28	2.12	2.00
MZB-L	0.66	-30.93	3.56	6.39
MZB-Z	2.55	-31.23	1.87	7.38
MZB-M	1.20	-33.64	1.99	0.14
ST-B	7.62	-28.26	5.76	6.02
ST-L	4.65	-28.66	3.98	2.27
ST-Z	6.68	-30.07	5.49	3.57
ST-M	5.50	-29.45	4.30	4.20
ST-AC1	9.84	-21.77	5.46	0.01
OM-AC2	11.39	-21.91	2.13	0.31
SL-AC3	11.07	-21.32	7.17	1.42
OM-AC4	10.67	-20.74	2.37	0.46

B, Bodingbach; L, Lassingbach; M, Mendlingbach; Z, Zellenbach; u, up; d, down, ST *Salmo trutta fario* , OM *Oncorhynchus mykiss*, SL *Salvelinus lepeschini*

To reveal a potential effect of nutrients added to fish farms on nearby streams we first performed analysis of variance to find out if there was a significant difference in metal and fatty acid concentrations between sampling stations upstream, aquacultures and downstream.

Analysis of variance revealed no significant differences in fish tissue ($p>0.05$) for MUFA, SAFA, n3-PUFA, n-6 PUFA, total FA, total lipid, and EFA (ARA, ALA, LIN, EPA, DHA) concentrations at the 4 sampling streams. Due to the limited sample size of benthic invertebrates we pooled the 4 sampling sites to be able to perform t-test analysis. There was no significant difference in total lipid or single essential fatty acid concentrations of macrozoobenthos between the upstream and downstream stations. Similarly, for all single

heavy metals – no significant difference among up, aquaculture and down could be revealed except for Zn (up-down: p= 0.03, AC-down: p=0.08) in Bodingbach.

Results of mixing models in streams showed that macrozoobenthos of all four streams relied mostly on phytobenthos (97.7 ± 1.1 %) and only used 2.3 ± 3.2% of the supplied feeds as a dietary source.

SIAR results in aquacultures showed that supplied feeds contributed 85.7 ± 1.2% to the diets of raised salmonids. The residual 14.3 ± 0.2% can be assigned to not identified diet sources, i.e. benthic invertebrates, that may enter the fish farm through the inflow or terrestrial insects.

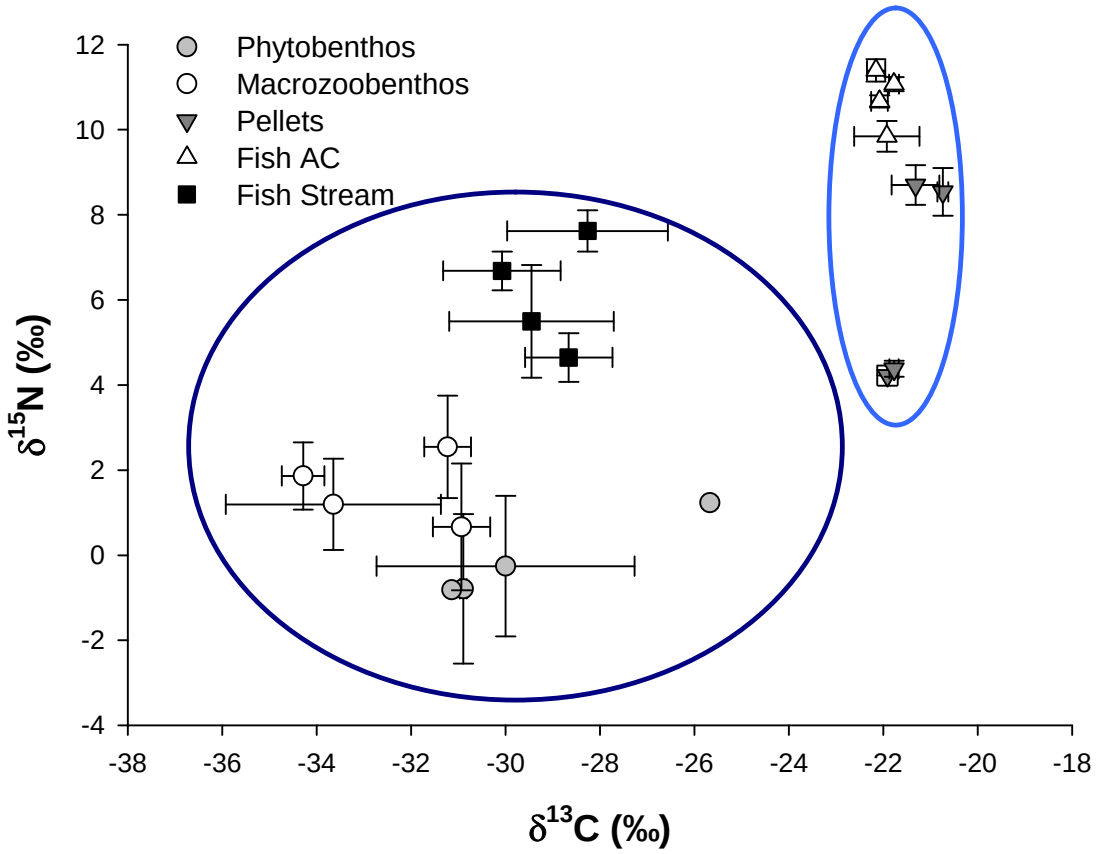

Figure 5 Stable isotope signatures of salmonids and pellets in aquaculture, salmonids, macrozoo- and phytobenthos in streams (stable isotope values down and upstream were pooled in rivers due to no significant difference). Circle dark-blue: Food Web Stream; circle light-blue: Food Web Aquaculture.

2.5. Discussion

2.5.1. Fatty acid and heavy metal profiles and their bioaccumulation in salmonids

Our study clearly showed that DHA was the mostly retained fatty acid in these freshwater salmonids of streams and aquaculture. Among PUFA, only DHA and ARA bioaccumulated in these fish. However, fatty acids, used as biochemical markers, have been recently used to better understand trophic relationships and energy transfers for aquatic organisms (e.g., Dalsgaard et al., 2003). In this study, dietary transfer of fatty acids and heavy metals from different diets to consumers was elucidated. Fatty acid composition of fish did not significantly differ, combining all sampling sites, among the two aquatic habitats except some essential fatty acids, namely ALA, ARA and DHA. In a previous study, Heissenberger et al. (2010) described no significant differences of FA concentrations among fish from pre-alpine streams.

Furthermore, concentrations of DHA in potential diets were much higher in aquaculture feeds than in macrozoobenthos. This explains the higher bioaccumulation rates in riverine salmonids and indicates that fish in these streams retain and/or convert DHA more efficiently than salmonids in nearby aquacultures that don't need to retain this essential fatty acid as there is enough provided in the added feed. However, dietary DHA is essential for the development and somatic growth of fish (Izquierdo et al. 2000; Copeman et al. 2002; Ballantyne et al. 2003). This underlies the physiological need of this essential fatty acid for fish in our studied aquatic ecosystems.

Despite the abundance of EPA and DHA in fish tissues, those are nearly the same in fish from streams and aquacultures, the importance of ARA as a primary eicosanoid precursor has been recognized in previous studies (Bell et al., 2003). Due to this physiological functional role its bioaccumulation behavior in aquaculture fish regarding the fact that EPA concentrations in pellets are significantly lower than in macrozoobenthos can be explained.

Heavy metal uptake of fish occurs mainly via water and food. In fish of these aquacultures, Mn and Se concentrations of pellet feeds were significantly correlated with concentrations of fish muscle tissues. Tracking the degree of Se in a given organism or tissue/organ (relative to Hg) is important for assessing ecotoxicological and human health risks because several

studies have demonstrated that this beneficial element acts to mitigate the toxic effects of Hg in organisms (Dietz et al., 2000). In certain quantities, Se, through various detoxifying mechanisms, may counteract Hg toxicity.

However, the higher Hg concentrations of riverine than aquaculture salmonids show that Hg bioaccumulation is higher in rivers than in these freshwater aquacultures. This can possibly be explained by the effect of somatic growth dilution (Jensen et al., 1982) in rapidly growing farmed fish. Whereas slower-growing, longer-lived wild fish of the same species and size as aquaculture-reared counterparts would carry higher individual Hg burdens due to their lower growth rates (Jardine et al., 2009). Moreover, comparison of BAFs (bioaccumulation factors) for different trophic links across the same set of sites normalizes the contribution of aqueous metal exposure, which can be assumed to be similar for different taxa at the same location (Besser et al., 2001).

Bioaccumulation factors for clearly defined trophic links (e.g., from benthic invertebrates or fish pellets to brook- and rainbow trout) reflect variation in the contribution of dietary metals, but not aqueous metals, to total metal bioaccumulation. In this study we observed that the only two metals that bioaccumulated in fish were Hg and Sn. Therefore, because of growth dilution in farmed fish, the bioaccumulation factors of the two metals reported here in farmed salmon are lower than those attained in mature wild fish.

For metals, which generally are non–fat soluble compounds, lipid contents have less influence on flesh residue concentrations compared to their concentrations in prey and their uptake and elimination kinetics (Kelly et al., 2008). This study elucidates that there is generally no relationship between total lipid and individual metal concentrations. However, the significant positive correlation between total lipids and Hg concentrations in fish does not seem to indicate a causal relationship because Hg is mostly bound to proteins (Campbell et al. 2005) and not related to lipids (Kainz et al. 2006). This stands in contrast with latest research. Qui et al. (2011) reported that Hg was the only metal not positively correlating with lipid content in aquaculture fish. Mercury is bioaccumulated as methyl Hg in organisms along the aquatic food chain (Chalmers et al., 2011; Wiener et al., 2006) and its elimination rates from fish are low (Ciardullo et al., 2008). Other metals, such as Cd, Pb, Ni, and Cu, generally are absorbed less efficiently and, hence, accumulate only slightly over time (Kelly et al., 2008). Therefore, when conducting heavy metal analysis in benthic invertebrates and fish single individual trace element kinetics, absorption and elimination, in different body

compartments should be considered. Muscle contributes the most mass to the whole body of a fish so whole body trace element concentrations in fish constitute a large mass in which trace element concentrations are regulated (Reinfelder et al., 1998). Furthermore, also Ciardullo et al. (2008) described muscle as the major storage site for MeHg. Hence, for most trace elements, whole tissues of invertebrates are much more responsive to exposure than whole tissues of fish (Reinfelder et al., 1998).

This study shows that neither farmed nor wild salmonids Hg or other trace metal concentrations exceeded federal consumption guidelines (Kommission der Europäischen Gemeinschaften, 2008; WHO, 1990).

2.5.2. Food Webs and the effect of aquaculture originated nutrients and potential contaminants on streams

In this study we find no evidence that aquacultures affect streamwaters. Aquatic food webs are generally affected by several factors: energy supply, environmental stability, ecosystem size, species composition and abiotic parameters. The elemental composition of biomass strongly depends on the nutritional conditions (Kuijper et al. 2004), therefore the nutritional value of food webs is determined by the biochemical composition of dietary nutrients including carbohydrate, proteins, lipids, and trace elements (e.g., Sterner and Elser 2002). Lipids are particularly important for aquatic consumers because some lipids are essential to their fitness and must thus be provided by food (e.g., Kainz et al. 2004). Trophic enrichment of $\delta^{15}N$ and $\delta^{13}C$ values in fish farms is strongly in line with our predicted two-trophic-level food chain (fish – added diet), where both, $\delta^{15}N$ and $\delta^{13}C$ values, were in the suggested range of 3.7 ‰ and <1.0‰ , respectively (Minagawa & Wada, 1984; Post, 2002). Therefore, our snapshot study was an approach to characterize two different types of food webs: the controlled two-trophic level food web of aquaculture and the more complex lotic food web.

Furthermore, results of stable isotope mixing models revealed that these freshwater salmonids fed mainly (~85%) on pellets. This clearly defined short trophic relationship in aquacultures opens a foundation for further analysis about the effect of diet to its consumer and to find out if fish are, biochemically, what they feed on.

The general trophic enrichment between brook trout and macrozoobenthos of the rivers was ~4‰, suggesting that fish had access to other, trophically intermediate prey items. Mainly $\delta^{13}C$ fractionation values indicate that in this study we did not consider other potential diet sources. Other studies suggested that salmonids are very active fish during daytime, are primarily drift feeders and choose their diet selectively (Moyle, 1977; Newman & Waters, 1984). Such diet possibly consists of smaller fish species and terrestrial insects (Hyvärinen & Huusko, 2006). Prey subsidies, such as adult aquatic insects, consist of mobile organisms that can cross-habitat boundaries (Polis et al., 1997). Results of heavy metals analysis support such missing trophic links. Mercury concentrations of riverine fish were significantly higher in riverine fish than in farmed fish, although Hg concentrations of their potential diets, i.e., benthic invertebrates and pellets, were very similar. Therefore, to define a clear riverine food web structure of these pre-alpine streams it is inevitable to regard more aspects than the three-step food chain approach (phytobenthos – macrozoobenthos – fish).

Since a fairly long time food web studies confirm that such structures are complex and that the high heterogeneity of riverine food webs and certain environmental impacts have a considerable influence on mass balance of stable isotopes (Power & Dietrich, 2002; Woodward & Hildrew, 2002). The study of stream and river food webs often has been limited to certain times of the year. This limitation could be a major barrier to understand stream food webs because temperate streams are open, changing systems in which trophic interactions and energy flow could be greatly affected by seasonal changes in potential food sources (Torrez-Ruiz et al., 2007). Hence a small sample size is also often insufficient to capture the true complexity of riverine food webs. When working on food webs of pre-alpine streams it is furthermore necessary to be aware about the base of these structures. Aquatic ecosystems are supported by endogenous carbon, which is produced by photosynthesis and autotrophic production of microbial communities (Thorp, 2002) and allochthonous carbon sources, which are produced elsewhere and transported as ie. leaf litter, sediment or even waste of anthropogenic origin into lotic ecosystems.

To understand if there was dietary uptake of fish pellets of fish farms by macrozoobenthos in recipient streams and so nutrients and potential contaminants may enter the riverine food web we performed mixing models to examine whether benthic invertebrates were feeding on aquaculture-derived feeds. If the concentration of any parameter downstream is very

similar to the parameters profile in aquacultures and significantly different to that upstream, a potential effect could be discussed. Our results clearly indicate that macrozoobenthos was mainly (~97%) feeding on autochthonous phytobenthos, and not on carbon or nitrogen input from aquacultures. In line with this finding, Rasmussen (2010) showed that algae are the dominant food source for herbivore/grazers, whereas shredders are supported largely by carbon derived from terrestrial sources and filterers and collectors feed on fine particulate matter, a mixture of terrestrial and aquatic detritus. Thorp (2002) also confirmed the importance of autochthonous primary production (algae) and explained that it is the major contributor to metazoan production in rivers**.** Therefore, from a nutritional point of view, we can exclude an effect of aquacultures on nearby streams. However, to be sure about all diet sources it would be essential to separate macrozoobenthos in its different feeding guilds.

This study provides evidence that fish farms don't cause changes in lipid- and heavy metal profiles of riverine organisms but other potential effects of aquaculture derived nutrients and contaminants, including i.e. changes in riverine species composition, should be investigated in future studies.

2.6. Conclusions

In this study we find no evidence that aquaculture derived nutrients or potential contaminants affect food webs of nearby streams. Furthermore we conclude that the diet of riverine salmonids consists not only of benthic invertebrates and that other diet sources should be investigated in future studies in pre-alpine streams.

Total lipid concentration did not significantly differ between fish from aquacultures and streams, whereas raised salmonids had higher DHA concentrations and riverine salmonids do have a higher content of ARA and ALA concentrations. Bioaccumulation was determined for DHA at all sampling sites, being aware of the higher bioaccumulation ratios in streamwater fish due to its limitation in potential feeds, and for ARA in 3 of 4 aquacultures. Therefore, we can confirm the physiological importance of DHA for fish.

In pre-alpine streams or aquacultures neither farmed nor wild salmonids Hg or other trace metal concentrations exceeded federal consumption guidelines. The only two heavy metals showing bioaccumulation in fish are mercury (Hg) and tin (Sn). This study elucidates that there is generally no relationship between total lipid and individual metal concentrations.

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3. Zusammenfassung und Perspektiven

Die Untersuchungen im Rahmen meiner Diplomarbeit ergaben zusammengefasst folgende Ergebnisse:

- Es gibt keinen signifikanten Unterschied hinsichtlich des Gesamtlipidgehalts und der PUFA-Konzentrationen zwischen Fischen in Aquakulturhaltung und jenen in Bächen. Es konnten jedoch folgende Unterschiede in den Profilen der essentiellen Fettsäuren festgestellt werden: Salmoniden aus Aquakulturanlagen haben einen höheren Gehalt an DHA, Salmoniden in Bächen weisen hingegen signifikant höhere ARA und ALA Konzentrationen auf.

- Bioakkumulation konnte nur für DHA nachgewiesen werden: Der Bioakkumulationsfaktor (BAF) ist in den Bächen höher als in den Aquakulturanlagen. Dies deutet auf die Notwenigkeit dieser essentiellen Fettsäure für physiologische Prozesse des Fisches hin.

- Quecksilber-, Eisen- und Selenkonzentrationen waren in den Fischen der Bäche signifikant höher als in Fischen der Aquakultur.

- Bioakkumulation zeigten nur Quecksilber (Hg) und fallweise Zinn (Sn). Hg reicherte sich in allen Untersuchungsstellen (AC und Bach) im Nahrungsnetz an, Sn hingegen nur an einigen Probenahmestellen.

- Die Schwermetallbelastung der untersuchten Salmoniden liegt weit unter den Richtwerten der Lebensmittelverordnung des UBA sowie der WHO und ist daher nicht als gesundheitsgefährdend zu erachten.

- Nahrungsnetzanalysen mittels stabiler Isotope konnten zeigen: Fische in der Aquakultur fressen zu über 90% das ihnen angebotene Fischfutter was sich auch in der Isotopensignatur zeigt: es konnte ein kontrollierbares Nahrungsnetz mit zwei trophischen Ebenen (Fischfutter – Fisch) nachgewiesen werden.

- Nahrungsnetze in natürlichen aquatischen Systemen sind weitaus komplexer als jenes dreistufige trophische Modell (Phytobenthos – Makrozoobenthos – Salmoniden) das in dieser Studie für Bäche angenommen wurde. Laut unseren Ergebnissen wurden andere potentielle Nahrungsquellen der Forellen nicht berücksichtigt.

- Es konnte kein Effekt der Aquakulturen auf die Lipid- und Schwermetallprofile in den Vorflutern festgestellt werden.

Nahrungsnetze in natürlichen lotischen Systemen sind viel komplexer als jene potentiell kontrollierbaren in Aquakulturanlagen. Dies konnte mit unseren Untersuchungen nur annähernd dargestellt werden. Es ist davon auszugehen, dass Salmoniden nicht nur Makrozoobenthos als Nahrungsgrundlage beziehen. Fischökologische Untersuchungen zeigen, dass Forellen auch andere kleine Fische, sowie Schnecken, Würmer und Anflugnahrung (terrestrische Insekten) fressen (Hyvärinen & Huusko, 2006).
Die Annahme, dass die Schwermetallkontamination proportional mit dem Fettgehalt des Fisches steigt konnte in dieser Studie nicht nachgewiesen werden.
Ein Vergleich der Lipid- und Schwermetallkonzentrationen im Fischgewebe zwischen einzelnen Bachstandorten (flussauf und flussab der AC) und den Aquakulturanlagen ergab keine signifikanten Unterschiede. Die Ergebnisse der Mixing Models, eine statistische Methode zur Berechnung der Herkunft der potentiellen Nahrung mit Hilfe der stabilen Isotope $\delta^{13}C$ und $\delta^{15}N$, konnten zeigen, dass benthische Invertebraten ausgespülte Futterpellets aus der Aquakultur, die sich in Biofilmen und epilithischen Algenmatten verfangen können, nicht als Nahrungsquelle nutzen. Folglich konnte kein eindeutiger Effekt, im Sinne von Nährstoff- und Schwermetalleintrag, der Aquakulturanlagen auf die nebenliegenden Bäche bewiesen werden.

Aquakulturanlagen, welche ihr Wasser aus umliegenden Flüssen und Bächen beziehen und dieses wieder in selbige zurückleiten, haben aber definitiv einen Einfluss auf ökologische Parameter und Strukturen der Fließgewässer. Einige Faktoren können hier eine große Rolle spielen: Der Anlage beigemengtes pelletiertes Kunstfutter, das im Idealfall zu 100% von den Zuchtfischen gefressen wird dürfte in beträchtlichen Mengen, direkt oder in gelöster Form, ausgeschwemmt werden. Auch Ausscheidungen der Fische in den Anlagen, potentielle Krankheitserreger und Antibiotika gelangen durch den Wasserrücklauf wieder in die umliegenden Flüsse.

Das österreichische Wasserrechtsgesetz inklusive einer speziellen Abwasseremissionsverordnung für Aquakulturanlagen, das Tierschutzgesetz, das Tierseuchengesetz und die Naturschutzgesetze der Länder sichern die Reinhaltung der Gewässer, eine gesunde und tiergerechte Produktion und geben die Rahmenbedingungen für die Fischerei in Österreich vor (vgl. ris.bka.gv.at).

Der Nationale Strategieplan Österreich im Rahmen des Europäischen Fischereifonds (vgl. ec.europa.eu) besagt:

- Der Sektor Aquakultur besteht in Österreich aus zwei verschiedenen Komponenten, der wassermengenbetonten Salmonidenproduktion und der flächenbetonten Teichwirtschaft von Karpfen und verschiedenen Nebenfischen.

- Der Schutz der Verbraucher ist durch zahlreiche gesetzliche Bestimmungen in Umsetzung des Gemeinschaftsrechtes hinlänglich gesichert. Die Information der Verbraucher z.B. über die ernährungsphysiologischen Vorteile von Fisch ist über geeignete Medien durchzuführen.

- Mit der Erlassung einer Abwasseremissionsverordnung für die Aquakultur (BGBl. II 397/2004) ist Österreich ein Vorreiter in Europa auf diesem speziellen Sektor des Gewässerschutzes.

- Schon derzeit besteht in kleinerem Rahmen eine Produktion von biologisch erzeugten Forellen und Karpfen unter der Marke „Biofisch“ bei Einhaltung strenger Richtlinien. Dieser Markt soll ausgebaut werden.

Zusammengefasst (laut FREILAND–Tierhaltungsstandards, 2008) sind folgende Kriterien für den „Biofisch“, dessen Nachfrage in Österreich stetig steigend ist, zu berücksichtigen:
Die Futtermittel müssen grundsätzlich aus Biologischer Landwirtschaft stammen. Um eine weitgehend artgemäße Aufzucht zu gewährleisten, erfolgt die Haltung der Fische angepasst an ihr natürliches Verhalten und ihre Bedürfnisse an den Lebensraum. Der Betrieb hat kontinuierlich eine Nährstoffbilanzierung zu erstellen und etwa notwendige Wasserreinigung so einzurichten, dass die Wasserqualität durch die Nutzung nicht unverhältnismäßig beeinträchtigt wird. Die Wassergüte- bzw. Gewässergüte darf sich durch die Nutzung zum Zwecke der Fischereiwirtschaft zwischen Ein- und Auslauf nicht verschlechtern bzw. es sind entsprechend wirksame Maßnahmen zu ergreifen.

Nun gilt es: will man den Gesamteinfluss auf Bäche und Flüsse quantifizieren und darstellen, so muss man sich a) überlegen welche biotischen und abiotischen Parameter der Aquakultur wiederum welche Parameter des Flusses verändern und b) welche Untersuchungen durchführt werden müssen um aussagekräftige Ergebnisse zu bekommen. Dafür ist es unumgänglich einen Referenzzustand festzulegen bzw. einen Flussabschnitt zu untersuchen, der definitiv außer Reichweite eines potentiellen Einflusses der Aquakultur liegt. Zur vollständigen Ermittlung der trophischen Zusammenhänge der aquatischen Organismen und der Struktur dieses Nahrungsnetz wären weitere Studien nötig so müsste der allochthone, terrestrische Kohlenstoffeintrag, trotz Widerlegung der Wichtigkeit dieser Kohlenstoffquelle für aquatische Ökosysteme (Brett et al., 2009) sowie der autochthone Abbau von Detritus durch mikrobielle Lebensgemeinschaften ermittelt werden. Weiters wäre eine genaue Unterteilung des Makrozoobenthos in Ernährungstypen (functional feeding groups: Weidegänger, Zerkleinerer, Filtrierer und Detritus- und Sedimentfresser) sowie eine Untersuchung der Isotopensignaturen der anderen Fischarten in diesen Gewässern sinnvoll.

Im Rahmen dieser Diplomarbeit und konnte mit den in Kapitel 2.3. angewendeten Methoden keine Aufnahme der in den Forellenzuchten verwendeten Fütterungsmittel durch das MZB in den Bächen nachgewiesen werden. Durch dieses Resultat sowie durch Lipid- und Schwermetallanalysen können wir eine Bioakkumulation der potentiell eingeschwemmten Nährstoffe in lotischen Nahrungsnetzen in den vier voralpinen Bächen in Niederösterreich ausschließen.

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5. Appendix

5.1. Zusammenfassung

Lipid-, Schwermetall- und stabile Isotopenanalysen von Salmoniden und deren potentieller Nahrung (Makrozoobenthos und Pellets) wurden in vier Voralpen-Bächen und Aquakulturanlagen in Niederösterreich durchgeführt. Die Ziele dieser Studie waren a) die Ermittlung der trophischen Positionen der Fische und der Nahrung in Bächen und Aquakulturanlagen b) der Vergleich von Fettsäuren- und Schwermetallprofilen und deren Bioakkumulationsverhalten in den zwei aquatischen Ökosystemen und c) die Feststellung eines potentiellen Effektes durch den Wasserrücklauf der Aquakulturanlagen auf die nebenliegenden Bäche.

Analysen mithilfe stabiler Isotope ($\delta^{13}C$ und $\delta^{15}N$) ließen unterschiedliche Isotopensignaturen der Fische in den Aquakulturen (-21.98 ± 0.17‰; 10.75 ± 0.67‰) und den Bächen (-29.12 ± 0.40‰; 6.13 ± 1.02‰) erkennen. Die berechneten Fraktionierungsfaktoren zeigten klar, dass Salmoniden in den AC ausschließlich die beigemengten Pellets fressen ($\Delta^{15}N$ 4.3‰, $\Delta^{13}C$ 0.55 ‰), hingegen die Nahrung der Fische in den Bächen aus benthischen Invertebraten und nicht identifizierter Nahrung bestand ($\Delta^{15}N$ 4.5‰, $\Delta^{13}C$ 3.4‰). Analysen der dorsalen Muskelgewebe ließen erkennen, dass die physiologisch wertvolle omega-3-PUFA, Docosahexaensäure (DHA; 22:6n−3) sowie Quecksilber (Hg) in allen Fischen der beiden aquatischen Ökosysteme bioakkumulierten.

Es konnten keine signifikanten Unterschiede der Gesamtlipidkonzentrationen zwischen AC und Bächen festgestellt werden (p=0.5). Konzentrationen der Arachidonsäure (ARA; 20: 4n-6; p<0.001) sowie alpha-Linolensäure (ALA; 18:3n-3; p=0.003) waren signifikant höher in Fischen der Bäche, DHA (p=0.04) in den Aquakulturen. Eisen (Fe), Quecksilber (Hg) und Selen (Se) -konzentrationen waren signifikant höher in Bächen (p<0.05).

Resultate der Mixing Models mit Stabilen Isotopen ($\delta^{13}C$, $\delta^{15}N$) deuten darauf hin, dass Nährstoffe und potentielle Kontaminanten aus Aquakulturen keinen Effekt auf Nahrungsnetze, sowie Schwermetall- und Fettsäureprofile der Fische in den Bächen haben.

5.2. Summary

Lipids, heavy metals, and stable carbon and nitrogen isotopes (SI) were examined in freshwater salmonids and their potential diets (macrozoobenthos and fishpellets) of 4 pre-alpine streams and aquacultures in Lower Austria. The aim of this study was to investigate (i) trophic position of fish and diet in streams and aquacultures, ii) comparing fatty acid and heavy metal profiles and their bioaccumulation patterns of the two aquatic habitats and to (iii) examine a potential effect of aquacultures in terms of nutrients and contaminants through the water discharge on nearby stream food webs.

Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) revealed different SI signatures for fish in aquacultures (-21.98 ± 0.17‰; 10.75 ± 0.67‰) and streams (-29.12 ± 0.40‰; 6.13 ± 1.02‰, respectively). Fractionation factors clearly showed that salmonids of aquacultures fed on pellets ($\Delta^{15}N$ 4.3‰, $\Delta^{13}C$ 0.55 ‰), whereas fish from streams fed on benthic invertebrates and other, not identified, diets ($\Delta^{15}N$ 4.5‰, $\Delta^{13}C$ 3.4‰). Results of dorsal muscle tissues analysis showed that the physiologically required omega-3 PUFA, docosahexaenoic acid (DHA; 22:6n−3) and the potentially toxic heavy metal mercury (Hg) were both mostly retained in all fish of all ecosystems. There were no significant differences of total lipid concentrations between AC and streams ($p=0.5$). In fish, arachidonic acid (ARA; 20: 4n-6; $p<0.001$) and α-Linolenic acid (ALA; 18:3n-3; $p=0.003$) concentrations were significantly higher in streams, however, DHA ($p=0.04$) in aquacultures. Iron (Fe), mercury (Hg) and selenium (Se) concentrations were significantly higher in stream ($p<0.05$). Results of stable isotope mixing models ($\delta^{13}C$, $\delta^{15}N$) indicated that aquaculture derived nutrients or heavy metals did not affect food webs of nearby streams.

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Çok teşekkür ederim!

5.4. Lebenslauf

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Weitere Kenntnisse

Sprachen	Englisch – sehr gute Kenntnisse Italienisch – gute Kenntnisse Latein – gute Kenntnisse Französisch – Grundkenntnisse Türkisch - Grundkenntnisse
EDV	Windows Office Paket, Sigma Plot, R, SPSS
Führerschein	B