

Linking brain size in wild stream-dwelling brown trout with dietary supply of omega-3 fatty acids

Libor Závorka¹, Magnus Lovén Wallerius¹, Martin J. Kainz¹, and Johan Höjesjö¹

¹Affiliation not available

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Abstract

1. Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) are key structural lipids and their dietary intake is essential for brain development of virtually all vertebrates. The importance of n-3 LC-PUFA has been demonstrated in clinical and laboratory studies, but little is known about how differences in availability of n-3 LC-PUFA in natural prey influence brain development of wild consumers. The availability of n-3 LC-PUFA in the prey communities is driven by primary producers and it is therefore distributed heterogeneously, but predictably across ecosystems, being higher in aquatic than in terrestrial food webs. Consequently, the numerous consumers foraging on the interface of aquatic and terrestrial food webs can differ substantially in their intake of n-3 LC-PUFA, which may lead to in brain development, yet, this hypothesis still remains to be tested.

2. Here we use the previously demonstrated shift towards higher reliance on n-3 LC-PUFA deprived terrestrial prey of native brown trout *Salmo trutta* living in sympatry with invasive brook trout *Salvelinus fontinalis* to explore this hypothesis.

3. We found that the content of n-3 LC-PUFA in muscle tissues of brown trout decreased with increasing consumption of n-3 LC-PUFA deprived terrestrial prey. Brain volume was positively related to content of the n-3 LC-PUFA, docosahexaenoic acid, in muscle tissues of brown trout.

4. Our study thus suggests that increased reliance on low quality diet of n-3 LC-PUFA deprived subsidies from terrestrial food web can have a significant negative impact on brain development of wild trout. These findings provide the first evidence of an intra-specific link between n-3 LC-PUFA content in natural prey and brain size of wild vertebrate consumers.

5. Ongoing global change is predicted to reduce the availability of dietary n-3 LC-PUFA across food webs. Therefore, our findings emphasise the need for further research on how wild consumers adapt to the shortage of dietary n-3 LC-PUFA in order to maintain optimal development and functioning of their brain, which is crucial for their fitness.

Abstract content goes here

Libor Závorka¹, Magnus Lovén Wallerius², Martin J. Kainz^{1*}, Johan Höjesjö^{2*}

¹WasserCluster Lunz – Inter-university Centre for Aquatic Ecosystem Research, A-3293, Lunz am See, Austria

²Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, SE-405 30, Gothenburg, Sweden

*Equal contribution to the manuscript

Key words

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Introduction

Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), in particular docosahexaenoic acid (DHA; 22:6n-3), are essential for optimal functioning of metabolism and neural development of most vertebrate consumers (Pilecky et al. 2021; Twining et al. 2021). DHA is highly retained by vertebrates (Pilecky et al. 2021) and it has been shown to be a dominant building block of brain of fishes (Ebm et al. 2021), birds (Speake & Wood 2005), and mammals (Innis 2007). Laboratory feeding studies have shown that increased dietary intake of n-3 LC-PUFA facilitates brain growth and neuron proliferation in rodents (Kawakita et al. 2006), passerine birds (Hall et al. 2014), and freshwater (Lund et al. 2012) and marine fishes (Ishizaki et al. 2001). These dietary induced changes in brain development have also been shown to influence cognitive skills of vertebrate consumers (Lund et al. 2012; Sugasini et al. 2017; Pilecky et al. 2021) and could thus potentially have a direct impact on their fitness in the wild (Boogert *et al.*, 2018). Yet, little is known about how n-3 LC-PUFA content in natural diets influences the development of vertebrate brain in the wild.

Primary producers drive the availability of dietary n-3 LC-PUFA in prey communities, which is generally lowest in terrestrial, intermediate in freshwater, and highest in marine food webs (Hixson et al. 2015; Brett et al. 2017; Colombo et al. 2017). Vascular plants dominating primary production in terrestrial ecosystems can synthesize only short chain n-3 PUFA alpha-linoleic acid (ALA, 18:3n-3), while multiple aquatic primary producers, especially microalgae, can synthesize long-chain n-3 PUFA *de novo*, mainly eicosapentaenoic acid (EPA; 20:5n-3), but also DHA (Arts et al. 2009; Brett et al. 2017; Twining et al. 2016). The dominant determinant of n-3 LC-PUFA synthesis by primary producers appears to be their phylogeny (Twining et al. 2016; Marzetz et al. 2017). Therefore, the structure of the primary producer community is determinant of the n-3 LC-PUFA availability to herbivores (Marzetz et al. 2017; Guo et al. 2018) and to consumers at higher trophic levels (Guo et al. 2021). Observational studies on wild European perch *Perca fluviatilis* (Scharnweber et al. 2021) and Eastern Phoebe *Sayornis phoebe* (Twining et al. 2019) have shown that diet of individuals from the same species can differ substantially in the content of n-3 LC-PUFA due to intra-specific niche partitioning (Araújo et al. 2011). Therefore, differences in diet quality in wild vertebrate consumers can potentially be large enough to cause intra-specific divergence in brain development, but this hypothesis remains to be tested.

Dietary ALA and EPA are utilized by many vertebrates for internal synthesis of DHA (Murray et al. 2014; Twining et al. 2021; Závorka et al. 2021). However, the synthesis of DHA from short-chain ALA requires more conversion steps and thus more energy than the synthesis from long-chain EPA (Pilecky et al. 2021; Twining et al. 2021). This energy trade-off can lead to the lower capacity of consumers feeding on diet deprived of n-3 LC-PUFA to synthesize DHA and retain it in the brain (Závorka et al. 2021; Pilecky et al. 2021). In addition to the diet quality, this energy trade-off can be amplified by intrinsic factors such as sex of individuals, as females may be able to retain less DHA in the brain due to the higher investment of n-3 LC-PUFA to gametes (Maklakov 2008; Hou & Fuiman 2020). The capacity to synthesize and retain DHA has also been shown to decrease over ontogeny in freshwater fishes (Chaguaceda et al. 2020) and mammals (Brenna 2011). A good example of the energetic trade-off linked to the synthesis of n-3 LC-PUFA from dietary sources of different quality are consumers that depend on a mix of prey from aquatic and terrestrial resources (Twining et al., 2019). Aquatic and terrestrial macroinvertebrates are reciprocal sources of energy and macronutrients (Nakano & Mukarami 2001; Sullivan et al. 2014), but aquatic macroinvertebrates provide substantially more long-chain EPA than terrestrial macroinvertebrates, which contain mainly short-chain ALA (Guo et al. 2018; Brett et al. 2019). Predominantly insectivorous freshwater fishes, such as stream salmonids, depend on macroinvertebrates from aquatic and terrestrial subsidies (Nakano & Mukarami 2001; Syrjänen et al. 2011; Sánchez-Hernández & Cobo 2018). Therefore, intra-specific dietary specialization and intrinsic capacity to synthesize and retain n-3 LC-PUFA in a freshwater fishes could provide a first general insight in how these dietary biomolecules influence brain development of vertebrates in the wild.

In this study, we focus on brown trout *Salmo trutta*, a salmonid native to European streams, which is a generalist consumer displaying a broad range of dietary specialization among populations (Sanchez-Hernandez 2020) and individuals (Evangelista et al. 2014). We use a river system where brown trout co-occurs with the invasive brook trout *Salvelinus fontinalis*, as previous studies have shown that brown trout increases the consumption of terrestrial macroinvertebrates (*i.e.*, low-quality prey deprived of n-3 LC-PUFA) in sympat-

try with invasive brook trout (Zavorka et al. 2017; Cucherousset et al. 2020; but see Horka et al. 2017). Therefore, this study system allows us to test an entirely natural setting how reduced dietary intake of n-3 LC-PUFA influence the physiology and brain development of wild consumers. We explore how dietary reliance on terrestrial prey affects brain size and brain morphology of brown trout in sympatry and allopatry with the invasive brook trout, while accounting for ontogenetic and sex differences among individuals. We predict that; *i*) higher reliance on terrestrial prey of brown trout reduces dietary intake of EPA; *ii*) the content of EPA and DHA in brown trout tissues decreases with increasing reliance on terrestrial prey; and *iii*) lower DHA content in brown trout tissues is associated with smaller brain size or changes in brain morphology (*i.e.*, region specific brain size reduction).

Material and Methods

Study sites and fish sampling

This study was conducted in three streams in the upper part of the catchment of the Viskan River, situated in southwest Sweden (57deg40'318"N, 12deg59'300"E; Appendix S1, Fig. S1). Brook trout was introduced to Sweden in 1892 (Aas et al. 2018), with the first electrofishing reports indicating a self-reproducing brook trout population in our study system dating to 1985 (SERS, 2013). While brook trout have occurred in European streams for over a century, its impact on the native species is still significant and it is recognized by many European countries including Sweden as an invasive alien species (*e.g.*, Strand et al. 2018). Populations of brook trout are established in the upstream sections of the sampled streams. Native brown trout occupy the whole system, and thus each stream contains an allopatric (brown trout only) and a sympatric (brown and brook trout co-occurring) section. The brown trout populations in the study stream are landlocked with no reported lake or seawards migration. Abundance of brown and brook trout in the studied streams ranges from 0.2 – 1 individual per meter of the stream length. At sympatric sites, brook trout is as abundant or more abundant than brown trout (Zavorka et al. 2017). Other fish species occurring in the study system are brook lamprey *Lamperta planeri*, common minnow *Phoxinus phoxinus*, and occasionally northern pike *Esox lucius*.

Experimental fish were collected by electrofishing (LR-20B; Smith-Root, Vancouver, WA, USA) carried out over a 500 m stretch at each study site (Appendix S1, Table S1) between May 11 and June 11, 2019 (Appendix S1, Table S1). All sampled brown trout were measured (fork length *i.e.*, from the tip of the snout to the end of the central caudal fin ray) and a small clip of the pelvic fin ($\sim 0.5 \text{ cm}^2$) was stored on ice and later in laboratory kept in freezer at -80 degC , awaiting stable isotope analysis. We have collected between 38 to 45 brown trout per sampling site with average fork length ranging from 93 to 143 mm (Appendix S1). Aquatic macroinvertebrates for fatty acid and bulk stable isotope analysis were collected using kick sampling and terrestrial macroinvertebrates were collected by hand-picking and dragging the kick net over the canopy surrounding the stream. The macroinvertebrate samples were collected at each sampling site the same day as the fish and were stored alive in an icebox and after determination in the laboratory kept frozen at -80 degC until further processing. Habitat quality was evaluated at each study site on July 10, 2019 using standardized protocol (Jonkopingsmodellen - Hallden et al. 2002, Appendix S1, Table S1) and photographs of habitats at each sampling site were taken (Appendix S1, Fig. S1). Consumption of aquatic macroinvertebrates by brown trout is known to increase with their abundance in the stream (Evangelista et al. 2014; Sanchez-Hernandez & Cobo 2018). Therefore, we performed a quantitative assessment of abundance and biomass of aquatic macroinvertebrates at each sampling site on July 11 and 12, 2019 during sunny stable weather, with no precipitation and low water flow. Aquatic macroinvertebrates were collected by a kick-net (30 x 25 cm, mesh size, 30 seconds of kicking at three spots within each study site representing all available habitats). Macroinvertebrates were counted and oven-dried at 60 degC for 48 hours and total dry biomass of macroinvertebrates at each sampling site was measured to the nearest 0.001 g (Appendix S1, Table S1).

Fish dissection

A subsample of brown trout (Table 1) at each study site was transported alive to the department of Biology

and Environmental Sciences at the University of Gothenburg for dissection. Before the dissection, fish were housed in holding tanks (30 L, 300 x 320 x 340 mm), which provided shelter structures (rocks and plastic plants) and fresh 12 degC water from a flow-through filtration system (flow rate: 2 L min⁻¹). Fish were not fed during this period. All fish were dissected within 20 hours after capture and to randomize the time effect, individuals from allopatric and sympatric sites were dissected in alternating order (with even and odd individuals from allopatry and sympatry respectively). Before dissection, fish were euthanized by an overdose of 2-phenoxyethanol (0.5 mL L⁻¹) and body mass and fork length were measured to the nearest 0.1 g and 1.0 mm respectively. Sex of individuals was determined by inspection of the gonads. Heads of fish were removed and fixed in 4% buffered (pH 6.9) paraformaldehyde solution. Brains were then dissected out as described in Gonda et al. (2009) by opening the skull along the anteroposterior axis and removing muscle tissue, nerves and bones until the brain could be lifted up from the skull and stored in 4% buffered paraformaldehyde solution until further procedure. Brains were imaged with a digital camera (EOS 40D with MP-E 65mm lens; Canon Inc., Tokyo, Japan). Images of brains were taken using the dorsal, left lateral and ventral views to calculate the total volume and the volume of cerebellum, optic tectum, telencephalon, olfactory bulb, and hypothalamus. Measurements were completed using ImageJ 1.48 (Schneider et al. 2021) and used to calculate volume with the formulas outlined by Pollen et al. (2007). Brain morphology was assessed by principal component analysis (Appendix S2) yielding two dominant principal components. PC1 was positively related to the volume of olfactory bulb, and hypothalamus and negatively related to cerebellum and optic tectum, while PC2 was positively related to the volume of telencephalon.

Fixing fish brains in paraformaldehyde solution in order to do morphological measurements prevented us from performing fatty acid analysis of the brain tissue. Therefore, we have used the muscle tissue as a proxy of n-3 LC-PUFA content in fish body. Muscles provide a good proxy for this purpose, because they represent the majority of fish biomass, and thus muscle metabolic activity and fatty acids content reflects individuals as a whole (Norin & Malte 2012; Gladyshev et al. 2018). In addition, biochemical composition of muscle tissue of salmonids has been shown to respond in similar direction to dietary deprivation of n-3 LC-PUFA as brain tissue, but with higher magnitude (Zavorka et al. 2021). Thus, samples (~1 g of wet mass) of dorsal and ventral muscle tissue samples were taken from the left side below the dorsal fin, above and below the lateral line respectively. Bones and skin residuals were mechanically removed from the tissue samples, before they were stored on dry ice and subsequently frozen at -80 degC.

Bulk stable isotope and fatty acids analysis

We used bulk tissue analyses of $\delta^{13}\text{C}$ fin clips and potential prey to estimate diet composition of experimental individuals. Freeze-dried and homogenized samples were analysed in WasserCluster Lunz, Austria. Isotope ratios are reported relative to the international Vienna PeeDee Belemnite carbonate standard. $\delta^{13}\text{C}$ values were mathematically corrected for lipid content following methods as described by Post et al. (2007). The baseline ^{13}C value was further corrected to account for variability in basal resources across the sampling sites (Olsson *et al.* 2009; Musseau et al. 2020) using the following equation:

$$\delta^{13}\text{C}_{\text{corr}i} = (\delta^{13}\text{C}_i - \delta^{13}\text{C}_{\text{inv}}) / \text{CR}_{\text{inv}}$$

where $\delta^{13}\text{C}_{\text{corr}i}$ is the corrected carbon isotopic ratio for individual i , $\delta^{13}\text{C}_i$ is the carbon isotopic ratio for individual i , $\delta^{13}\text{C}_{\text{inv}}$ is the average carbon isotope ratio of macroinvertebrates and CR_{inv} is the carbon range ($\delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$) of macroinvertebrates. $\delta^{13}\text{C}_{\text{corr}i}$ is hereafter used as an indicator of reliance on terrestrial prey (*i.e.*, increasing value suggests increasing reliance of an individual on terrestrial prey).

Fatty acids were extracted and analysed from freeze-dried samples (3–10 mg dry mass) that were homogenized, sonicated and vortexed (4 times) in a chloroform-methanol (2:1) mixture, following Böhm et al. (2014). Total lipid mass ratios were determined via gravimetry. Fatty acids were derivatized to obtain fatty acid methyl esters (FAME) using toluene and sulfuric acid-methanol-solution (incubated at 16 h at 50 C). FAME were identified using a gas chromatograph (Thermo Scientific TRACE GC Ultra) equipped with a flame ionization detector (FID) and a Supelco SP-2560 column (100 m, 25 mm i.d., 0.2 mm film thickness). Quantification of fatty acids were performed by comparison with a known concentration of the internal

standard using Excalibur 1.4 (Thermo Electron Corporation).

Statistical analysis

All analyses were conducted in R v4.0.2 (<http://www.R-project.org/>). The structure of the final linear models and sample sizes break down by the levels of categorical explanatory variables are summarized in (Appendix S3). Non-significant interactions were removed from the models. Significance of the final models was evaluated using ANOVA tables using Type II and III sums of squares for models without and with significant interaction, respectively. Differences among groups were analysed using Tukey’s HSD post-hoc test. We have accounted for the ontogenetic effects by using fork length as a proxy of individuals age. Fork length has been used as explanatory variable in all models. When models contained other explanatory variables correlated to the fork length, we used residuals from a linear model between the log transformed variable of interest and fork length to prevent collinearity (see Appendix S3).

We tested the variability in content of ALA and EPA, and of total lipids in macroinvertebrates across all sampling sites (*i.e.*, category with 6 levels – 3 streams × 2 populations [allopatric, sympatric]) and subsidy types (category with 2 levels – aquatic or terrestrial macroinvertebrates). Since there was no difference lipid quality of aquatic and terrestrial subsidies across the six sampling sites (see results for details), we did not include the stream or sampling site in the models. We collected samples of dorsal and ventral muscle tissue because concentration of adipose cells is usually higher in ventral than in dorsal muscles and thus these two tissues can differ in their lipid content (Fauconneau et al. 1995; Ebm et al. 2021). However, the total lipid content and the relative content of n-3 LC-PUFA were repeatable across dorsal and ventral muscle samples (total lipids: $R_{\text{adj}} = 0.25$, 95% CI [0.06, 0.44]; ALA: $R_{\text{adj}} = 0.59$, 95% CI [0.44, 0.72]; EPA: $R_{\text{adj}} = 0.62$, 95% CI [0.48, 0.74]; DHA: $R_{\text{adj}} = 0.56$, 95% CI [0.41, 0.70]). Therefore, for further analysis we have used an average value across tissues for each individual. Total lipids in all models were reported as mass fractions (mg/g of the dry mass), while all models and figures including n-3 LC-PUFA reported the relative fatty acid contents (% of fatty acid methyl esters; with exception of Fig. 1). This is a common approach (*e.g.*, Twining et al. 2019; Ebm et al. 2021) because mass fractions of fatty acids are often closely correlated to the total lipid mass fractions, while the relative content of fatty acids is independent of the total lipids and thus more comparable across the sample types and studies, particularly when only a part of an organism (*e.g.*, muscle tissues) is analysed.

Results

Reliance on terrestrial prey was higher in sympatric than in allopatric brown trout ($F_{1;161} = 52.67$, $p < 0.001$; Fig. 1). The reliance on terrestrial prey also increased with increasing body size ($F_{1;161} = 98.26$, $p < 0.001$), but it did not differ between the sexes ($F_{1;79} = 0.01$, $p = 0.911$; Fig. 1). Reliance on terrestrial prey increased with increasing abundance ($F_{1;161} = 120.01$, $p < 0.001$) and biomass ($F_{1;161} = 63.06$, $p < 0.001$) of aquatic macroinvertebrates at the sampling site. This indicates that the consumption of aquatic and terrestrial prey by brown trout was not limited by the availability of aquatic prey in the stream, but abundance and biomass of aquatic macroinvertebrates appeared to be controlled by the foraging behaviour of brown trout.

Terrestrial macroinvertebrates contained less total lipids (mean±SD = 148±69 mg/dry mass) than aquatic prey macroinvertebrates (mean±SD = 204±86 mg/g dry mass; $F_{1;51} = 8.93$, $p = 0.004$). Terrestrial macroinvertebrates had similar content of ALA ($F_{1;51} = 1.39$, $p = 0.245$) and significantly lower EPA ($F_{1;51} = 30.27$, $p < 0.001$) than aquatic macroinvertebrates (Fig 1a). Total lipids ($F_{5,55} = 1.70$, $p = 0.151$), and the content of ALA ($F_{5,55} = 0.40$, $p = 0.845$) and EPA ($F_{5,55} = 1.00$, $p = 0.42$) of macroinvertebrates did not differ across the sampling sites. Aquatic macroinvertebrates were thus a richer dietary source of n-3 LC-PUFA, particularly of EPA, than terrestrial macroinvertebrates across all sampling sites (Fig. 1a).

The content of total lipids in brown trout tissues did not differ between sympatric and allopatric populations ($F_{1,80} = 0.06$, $p = 0.803$) and was not significantly related to the reliance on terrestrial prey ($F_{1,80} = 0.02$, $p = 0.890$), fork length ($F_{1,80} = 2.23$, $p = 0.139$), and sex ($F_{1,80} = 0.07$, $p = 0.789$) of individuals. The ALA content in trout tissues was affected by an interaction between the competition mode and reliance on

terrestrial prey ($F_{1,79} = 5.71$, $p = 0.019$; Fig. 1b), so ALA increased with increasing reliance on terrestrial prey in sympatric ($F_{1,39} = 8.74$, $p = 0.005$), but not in allopatric ($F_{1,38} = 0.73$, $p = 0.397$) brown trout. Sex ($F_{1,79} = 0.52$, $p = 0.472$) and body length ($F_{1,79} = 3.14$, $p = 0.080$) had no significant effect on ALA content in trout tissues. The EPA content was higher in allopatric than in sympatric brown trout ($F_{1,80} = 14.29$, $p < 0.001$; Fig. 1c) and decreased with increasing fork length ($F_{1,80} = 146.89$, $p = 0.001$), but was not affected by individual's sex ($F_{1,80} = 0.28$, $p = 0.597$) and reliance on terrestrial prey ($F_{1,80} = 0.87$, $p = 0.353$). The DHA content in trout tissues was affected by an interaction between the competition mode and reliance on terrestrial prey ($F_{1,78} = 7.46$, $p = 0.007$; Fig. 1d), so DHA decreased with increasing reliance on terrestrial prey in sympatric ($F_{1,38} = 7.14$, $p = 0.011$), but not in allopatric ($F_{1,38} = 1.07$, $p = 0.307$) populations. Sex had no effect on DHA in trout tissues ($F_{1,78} = 0.74$, $p = 0.391$), but DHA content decreased with increasing body size ($F_{1,78} = 6.33$, $p = 0.014$).

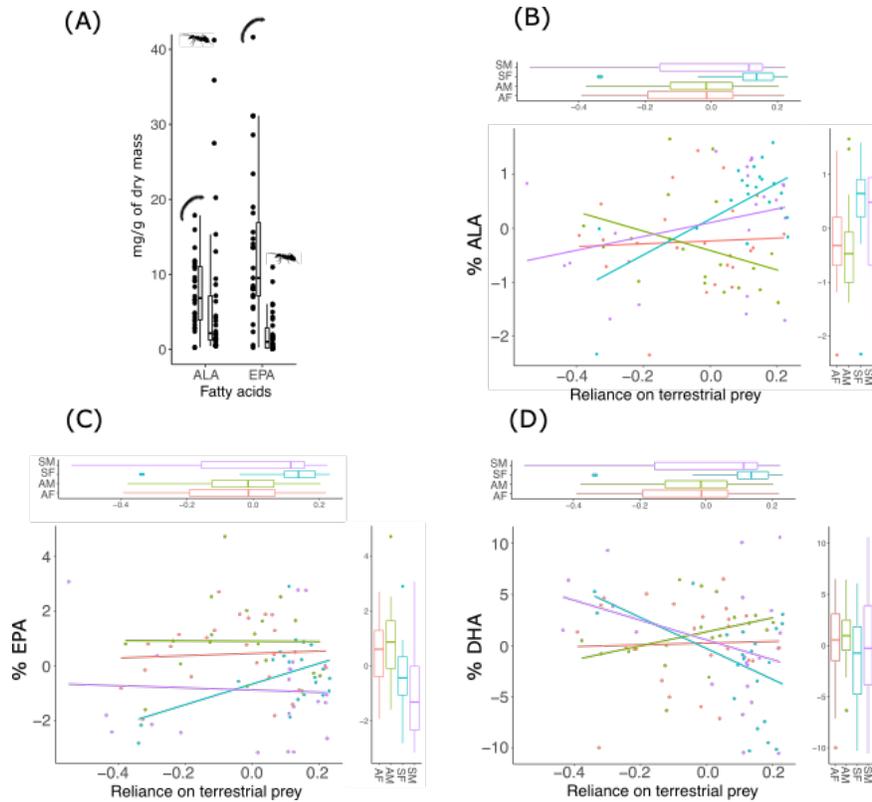


Figure 1 – (a) Content mg/g of ALA and EPA in aquatic and terrestrial prey macroinvertebrates. All prey samples contained no or negligible amount of DHA. Association of the reliance on terrestrial prey and the relative content of ALA (b), EPA (c), and DHA (d) in trout tissues. Colours of datapoints, fit lines and box plots displaying distribution of each variable correspond to AF – allopatric females (red), AM – allopatric males (green), SF – sympatric females (cyan), and SM – sympatric males (purple). Variables in figures b-d are residuals controlled for the fork length of individuals.

Total brain volume increased with increasing DHA content in trout tissues ($F_{1,80} = 11.57$, $p = 0.001$, Fig. 2a), but DHA content had no effect on brain morphology (PC 1: $F_{1,80} = 0.92$, $p = 0.341$, Fig. 2b; PC2: $F_{1,80} = 3.89$, $p = 0.052$, Fig. 2c). Total lipids content in trout tissues had no significant effect on total brain volume ($F_{1,81} = 2.92$, $p = 0.092$) and morphology (PC 1: $F_{1,81} = 1.27$, $p = 0.263$; PC2: $F_{1,81} = 3.94$, $p = 0.051$). The brain volume increased with increasing fork length of individuals ($F_{1,80} = 657.45$, $p < 0.001$), but brain morphology was independent of individual fork length (PC 1: $F_{1,81} = 0.07$, $p = 0.786$; PC2: $F_{1,80}$

= 0.01, $p = 0.943$). Brain volume and brain morphology did not differ between allopatric and sympatric populations (brain volume: $F_{1,80} = 0.51$, $p = 0.476$; PC 1: $F_{1,80} = 0.99$, $p = 0.322$; PC2: $F_{1,80} = 0.00$, $p = 0.990$) or between the sexes (brain volume: $F_{1,80} = 1.54$, $p = 0.219$; PC 1: $F_{1,81} = 0.57$, $p = 0.454$; PC2: $F_{1,80} = 0.05$, $p = 0.943$).

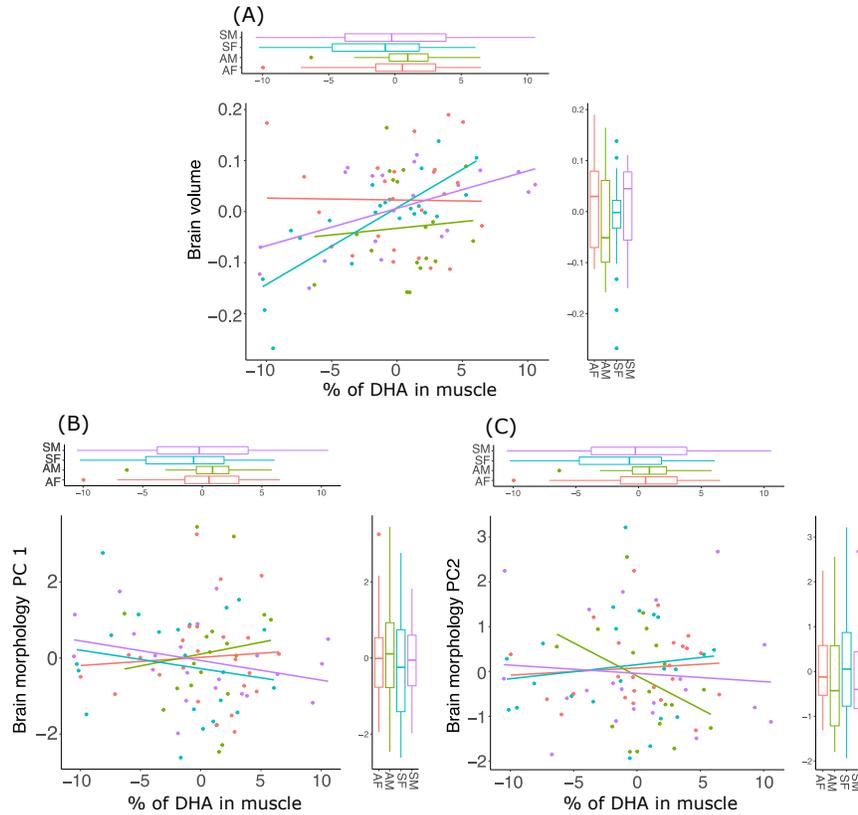


Figure 1: **Figure 2** – Association between DHA in trout tissues and the brain volume (a), brain morphology PC1 [PC1 was positively related to the volume of olfactory bulb, and hypothalamus and negatively related to cerebellum and optic tectum] (b) and brain morphology PC2 [PC2 was positively related to the volume of telencephalon] (c). Colours of datapoints, fit lines and box plots displaying distribution of each variable correspond to AF – allopatric females (red), AM – allopatric males (green), SF – sympatric females (cyan), and SM – sympatric males (purple). All variables are residuals controlled for the fork length of individuals.

Discussion

Dietary reliance of brown trout on low-quality terrestrial prey, deprived of n-3 LC-PUFA, increased in sympatry with invasive brook trout. Higher reliance on terrestrial prey of sympatric brown trout resulted in lower content of EPA in their muscle tissues compare to the allopatric conspecifics. In the sympatric populations, the content of the short-chain ALA increased and the content of the long-chain DHA decreased in muscle tissues with increasing reliance on the low-quality terrestrial prey, but the relative content of ALA and DHA in allopatric brown trout was not related to the diet quality. Sex had no effect on diet quality and muscle content of n-3 LC-PUFA, but larger (and presumably older, *i.e.*, Bowker 1995; Öhlund et al. 2008) individuals relied more on terrestrial prey and consequently had lower relative content of n-3 LC-PUFA in muscles. We found that the relative content of DHA in muscle tissues was positively related to the encephalization of brown trout (*i.e.*, higher relative brain size after controlling for the body size). These findings demonstrate that intra-specific differences in diet quality in wild vertebrates, foraging at interface

of aquatic and terrestrial food webs, are linked to their brain size. In addition, our findings indicate that external stressors, such as competition with an invasive species, can reduce the capacity of consumers to synthesize and retain DHA for brain development.

Dietary intake and retention of energy in form of lipids has a substantial effect on the physiological development of consumers (Arts et al. 2009). However, our results indicate, in agreement with previous laboratory studies (Speake & Wood 2005; Lund et al., 2012; Hall et al. 2014), that the availability of structural lipids, especially DHA, and not the lipid energy reserve is the key for brain development in vertebrate consumers. Our findings suggests that the specialization of dietary niche in wild consumers on resources that have similar content of energy, but differ in the content of n-3 LC-PUFA (Heissenberger et al. 2010; Twining et al. 2019; Scharnweber et a. 2021), can lead to intra-specific diversification of brain size. This is an important finding because brain size is positively correlated to cognitive capacity, especially among closely related species (Olkowicz et al. 2016) and at the intra-specific level (Møller 2010; Kotrschal et al. 2013). For example, guppies with larger brain size have shown higher learning capacity (Kotrschal et al. 2013), and large brain have been suggested to improve migration efficiency and offspring defence in barn swallows *Hirundo rustica* (Møller 2010). However, it should be noted that while dietary supply of omega-3 LC-PUFA is determinant for brain development of vertebrates (Pilecky et al. 2021), dietary availability of these biomolecules can also influence other phenotypic traits important for consumers fitness, such as mitochondrial efficiency (Salin et al. 2021; Závorka et al. 2021); metabolic rate (McKenzie 2001; Twining et al. 2016), growth rate (Chaguaceda et al. 2020; Závorka et al. 2021), and reproductive capacity (Brett et al. 2009; Scharnweber & Gårdmark 2020). Therefore, further carefully designed experiments in a realistic ecological context are needed to understand the trajectory of how dietary omega-3 PUFA influences fitness of consumers in the wild.

Effects of diet quality on development of phenotypic traits necessarily depends on temporal scales on which the consumption of low- and high-quality diets occurs (Brett et al. 2009; Murray et al. 2014). For example, a relatively short-term study (*i.e.*, 8 weeks of dietary treatment) by Závorka et al. (2021) on juvenile Atlantic salmon *Salmo salar* showed a strong shift of DHA content in the brain, but no significant effect on brain size and performance in a cognitive test. On the other hand, a longer study (*i.e.*, 21 weeks of dietary treatment) by Lund et al. (2012) on juvenile pikeperch *Sander luciperca* demonstrated that dietary n-3 LC-PUFA deprivation induces leads to reductions of DHA content and size of the brain resulting in lower cognitive capacity. The reliance on terrestrial prey in our study was estimated based on bulk $\delta^{13}\text{C}$ values of fin clips, which indicate dietary carbon on the temporal scale of several weeks (Jardine et al. 2005; Layman et al. 2012). However, a previous study has shown that the dietary shift of our focal species, brown trout, in sympatry with invasive brook trout occurs early in ontogeny and remains stable in later life-stages (Cucherousset *et al.*, 2020). The increased reliance on low-quality terrestrial prey in our study did not appear to be influenced by the availability of aquatic prey, indicating that this dietary shift was likely caused by behavioural changes of brown trout in sympatry with the invasive species (Lovén Wallerius et al. 2017; Larranaga et al. 2018; Cucherousset *et al.*, 2020). Thus, the intra-specific dietary differences in our study likely represent a long-term specialization of individuals that might have a permanent effect on supply of n-3 LC-PUFA to their brain.

Despite the higher reliance on low-quality terrestrial prey and corresponding decrease of EPA in muscle tissues, there was no significant reduction of DHA in muscles of sympatric compared to allopatric brown trout. A potential explanation is that sympatric brown trout has adapted to the lower dietary intake of n-3 LC-PUFA by increasing retention and/or internal synthesis of DHA (Murray et al. 2014). Such adaptation has been observed in populations of three-spined stickleback (Ishikawa *et al.*, 2021) and European perch (Scharnweber *et al.*, 2021) foraging on low-quality prey from littoral lake habitat that is deprived of n-3 LC-PUFA. Similarly, tree swallows have been shown to increase synthesis of DHA when consuming n-3 LC-PUFA deprived terrestrial macroinvertebrates (Twining *et al.*, 2018). However, our results indicated limits of this adaptation as the relative content of DHA in sympatric trout decreased with increasing reliance on terrestrial prey. This suggests that the combination of external stressor (*i.e.*, interaction with an invasive species) and high consumption of n-3 LC-PUFA deprived prey can affect the capacity of wild consumers to

maintain an optimal biochemical composition of their tissues. Synthesis of DHA from short-chain precursors, such as ALA, contained in low-quality terrestrial diet comes at substantial energetic costs that can result in reduced somatic growth rate (Murray et al. 2014; Twining et al. 2016; 2018; Závorka et al. 2021). Our findings thus accentuate the suggestion that the trade-off between internal synthesis and dietary acquisition of n-3 LC-PUFA might be an important driver of phenotypic adaptations across vertebrate consumers (Twining et al. 2021). We also found that larger (and presumably older, *i.e.*, Bowker 1995; Öhlund et al. 2008) individuals across populations relied more on terrestrial prey and had lower EPA and DHA contents in muscles. This age-dependent effect can be explained by the fact that older consumers require less n-3 LC-PUFA than younger consumers (Brenna 2011; Chaguaceda et al. 2020), because the key period of vertebrate brain development occurs during early ontogenetic stages (Innis 2007; Brenna 2011; Lund et al. 2012).

In conclusion, this field study demonstrates the largely overlooked importance of dietary intake of n-3 LC-PUFA for brain development of wild vertebrate consumers, which have so far been recognized just under laboratory conditions (Pilecky et al. 2021). We have shown the link between n-3 LC-PUFA deprived diets and consumer's brain size in the context of competition with an invasive species. However, the ongoing global change is predicted to limit availability of n-3 LC-PUFA for vertebrate consumers on a large spatial scale (Hixson & Arts, 2016; Heilpern et al. 2021) and it is still poorly understood how wild vertebrate consumers respond to this dietary deprivation of n-3 LC-PUFA. Our results indicate that it can have negative impacts even on species which are pre-adapted to dietary scarcity of these important biomolecules (*i.e.*, salmonids often consume high proportion of low-quality terrestrial prey, Syrjänen et al. 2011; Evangelista et al. 2014). Dietary intake of n-3 LC-PUFA have a high potential to affect fitness of vertebrate consumers (Twining et al. 2021; Pilecky et al. 2021), and therefore further studies are needed to understand how the availability n-3 LC-PUFA in natural diets affects development of brain, behaviour, physiology and life-history of vertebrates.

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Data archiving

Should the manuscript be accepted data will be archived at figshare.com (doi:) and in Supplementary material.

Ethics statement

These experiments were approved by the Ethical Committee for Animal Research in Göteborg (license dnr 5.8.18-04106/2018) and comply with current laws in Sweden.

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