Linking omega-3 polyunsaturated fatty acids in natural diet with brain size of wild consumers

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Abstract
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 the availability of n-3 LC-PUFA in natural prey influence brain development of wild consumers. Consumers foraging at the interface of aquatic and terrestrial food webs can differ substantially in their intake of n-3 LC-PUFA, which may lead to differences in brain development, yet this hypothesis remains to be tested. 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. 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 the content of the n-3 LC-PUFA, docosahexaenoic acid, in muscle tissues of brown trout. Our study thus suggests that increased reliance on diets low in n-3 LC-PUFA, such as terrestrial subsidies, can have a significant negative impact on brain development of wild trout. Our findings provide the first evidence of how brains of wild vertebrate consumers response to scarcity of n-3 LC-PUFA content in natural prey.

Keywords Essential fatty acids · Neurogenesis · Encephalization · Animal cognition · Ecosystem functioning · Biological invasions

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. 2021a). DHA is highly retained by vertebrates (Pilecky et al. 2021) and it has been shown to be a dominant building block of brains in fishes (Ebm et al. 2021), birds (Speake and 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 influenced cognitive skills of vertebrate consumers (Lund et al. 2014; 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 brains 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 (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 n-3 LC-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 of 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 endogenous DHA synthesis (Murray et al. 2014; Twining et al. 2021a; 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. 2021a). This energy trade-off can lead to 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 not be able to retain as much DHA in the brain due to the higher investment of n-3 LC-PUFA to gametes compared to males (Maklakov et al. 2008; Hou and Fuiman 2020). The capacity to synthesize and retain DHA has also been shown to decrease during 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 qualities 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 and Murakami 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. 2017). Predominantly insectivorous freshwater fishes, such as stream salmonids, depend on macroinvertebrates from aquatic and terrestrial subsidies (Nakano and Murakami 2001; Syrjänen et al. 2011; Sánchez-Hernández and Cobo 2018). Therefore, intra-specific dietary specialization and intrinsic capacity to synthesize and retain n-3 LC-PUFA in freshwater fishes could provide first general insight into 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 (Sánchez-Hernández 2020) and individuals (Evangelista et al. 2014). We use a river system where brown trout increase their consumption of terrestrial macroinvertebrates (i.e. low-quality prey deprived of n-3 LC-PUFA) when in sympathy with invasive brook trout *Salvelinus fontinalis* (Cucherousset et al. 2007; Závorka et al. 2017; but see Horká et al. 2017). Therefore, this study system allows us to test in a natural setting how reduced dietary intake of n-3 LC-PUFA influences the physiology and brain development of wild consumers. We explore how dietary reliance on low-quality terrestrial prey affects brain size and brain morphology of brown trout in sympathy and allopatry with 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).

**Materials 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 (57° 40’ 318” N, 12° 59’ 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 (Šers 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. https://www.havochvatten.se/arter-och-livsmiljoer/frammande-arter/sok-frammande-arter/fakta/backgounding.html 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 streams are landlocked with no reported lake or seawards migration. Abundance of brown and brook trout in the studied streams ranges from...
0.2 to 1 individual per meter of the stream length. At sympatric sites, brook trout are as abundant or more abundant than brown trout (Závorka et al. 2017). Other fish species occurring in the study system are brook lamprey Lampetra planeri, common minnow Phoxinus planarius, 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 (~0.5 cm²) was stored on ice and later in laboratory kept in freezer at −80 °C for 48 h and total dry biomass of macroinvertebrates (Appendix S1, Table S1). Macroinvertebrates were counted and oven dried at 60 °C. 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 °C until further processing. Habitat quality was evaluated at each study site on July 10, 2019, using a standardized protocol (Jönköpingsmodellen—Halldén et al. 2002, Appendix S1, Table S1), and photographs of habitats at each sampling site were taken (Appendix S1, Fig. S1). Aquatic macroinvertebrates by brown trout is known to increase with their abundance in the stream (Evangelista et al. 2014; Sánchez-Hernández and 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 (30×25 cm, mesh size, 30 s of kicking at three spots within each study site representing all available habitats). Macroinvertebrates were counted and oven dried at 60 °C for 48 h 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 (Appendix S1, Table S1) at each study site was transported alive to the department of Biology and Environmental Sciences at the University of Gothenburg. Fish were housed in holding tanks (30 L, 300×320×340 mm), which provided shelter structures (rocks and plastic plants) and fresh 12 °C water from a flow-through filtration system (flow rate: 2 L min⁻¹). Fish were not fed during this period. All fish were subsequently dissected within 20 h after capture and, to randomize the time effect, individuals from allopatric and sympatric sites were dissected in alternating order. Before dissection, fish were euthanized by an overdose of 2-phenoxethanol (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, 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 65 mm 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 for morphological measurements prevented us from performing fatty acid analysis of the brain tissue. Therefore, we 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 acid content reflect individuals as a whole (Norin and Malte 2012; Gladyshev et al. 2018). In addition, the biochemical composition of muscle tissue of salmonids has been shown to respond similarly to dietary deprivation of n-3 LC-PUFA as brain tissue, but with higher magnitude (Závorka et al. 2021). 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. 1997; Ebm et al. 2021). Therefore, 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 °C.
**Bulk stable isotope and fatty acids analysis**

We used bulk tissue analyses of $\delta^{13}$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. $\Delta^{13}$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:

$$\delta^{13}C_{corr} = \frac{\delta^{13}C_{i} - \delta^{13}C_{inv}}{CR_{inv}},$$

where $\delta^{13}C_{corr}$ is the corrected carbon isotopic ratio for individual $i$, $\delta^{13}C_{i}$ the carbon isotopic ratio for individual $i$, $\delta^{13}C_{inv}$ the average carbon isotopic ratio of all macroinvertebrates (i.e. terrestrial and aquatic included), and $CR_{inv}$ is the carbon range (δ13Cmax − δ13Cmin) of all macroinvertebrates, 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 sulphuric 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 μm 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 linear mixed effects models and sample sizes breaking down by the levels of categorical variables are summarized in Appendix S3. We included stream (category with 3 levels) as a random intercept in all models. Non-significant variables were removed step wise from the models with non-significant interactions removed in the first step and non-significant main effects removed in the second step. No tested interaction (Appendix S3) was significant and thus not reported. Significance of the models was evaluated using ANOVA tables using Type II and III sums of squares for models without and with 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 fixed effects in all models. When models contained other fixed effects correlated to the fork length, we used residuals from a linear regression between the log transformed variable of interest and fork length to prevent collinearity (see Appendix S3). We found a clear outlying value in the models of brain volume, and therefore, we report results of the analysis with and without the outlier.

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). The total lipid content and the relative content of n-3 LC-PUFA were repeatable across dorsal and ventral muscle samples (total lipids: $R_{adj} = 0.25$, 95% CI [0.06, 0.44]; ALA: $R_{adj} = 0.59$, 95% CI [0.44, 0.72]; EPA: $R_{adj} = 0.62$, 95% CI [0.48, 0.74]; DHA: $R_{adj} = 0.56$, 95% CI [0.41, 0.70]). Therefore, for further analysis we used an average value across tissues for each individual. Total lipids in all models were reported as mass fractions (mg/g dry mass), while all models, including n-3 LC-PUFA, used the relative fatty acid contents (% of fatty acid methyl esters). This is a common approach (e.g. Twinning 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 less dependent on 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 overall higher in sympatric than in allopatric brown trout ($\chi^2 = 30.87$, $p < 0.001$; Fig. 1). The reliance on terrestrial prey also increased with increasing body size ($\chi^2 = 71.37$, $p < 0.001$; Fig. 1), but it did not differ between the sexes ($\chi^2 = 0.48$, $p = 0.490$). Reliance on terrestrial prey increased with increasing abundance ($\chi^2 = 24.27$, $p < 0.001$) and biomass ($\chi^2 = 28.17$, $p < 0.001$) 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 macroinvertebrates in the stream, but abundance and biomass of aquatic macroinvertebrates appeared to be controlled by the foraging behaviour of brown trout.

Terrestrial macroinvertebrates contained on average less total lipids (mean ± SD = 148 ± 69 mg/dry mass) than aquatic prey macroinvertebrates (mean ± SD = 204 ± 86 mg/g
dry mass; $F_{1,51} = 8.93$, $p = 0.004$). Across all sampling sites, 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. 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 among 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.

The content of total lipids in brown trout tissues was not significantly related to the reliance on terrestrial prey ($\chi^2 = 1.92$, $p = 0.166$). The content of total lipids did not differ between sympatric and allopatric populations ($\chi^2 = 0.16$, $p = 0.69$) or sexes ($\chi^2 = 0.32$, $p = 0.571$), but it increased with increasing fork length ($\chi^2 = 3.91$, $p = 0.048$). The ALA content was not significantly related to the reliance on terrestrial prey on terrestrial prey ($\chi^2 = 1.88$, $p = 0.170$; Fig. 2a), but it was significantly higher in sympatric than allopatric brown trout ($\chi^2 = 14.73$, $p < 0.001$; Fig. 2a). The ALA content increased with increasing fork length ($\chi^2 = 7.50$, $p = 0.007$) and total lipid content ($\chi^2 = 9.34$, $p = 0.002$) of individuals, but it did not differ between sexes ($\chi^2 = 1.57$, $p = 0.211$, Fig. 2a). The EPA content decreased with increasing reliance on terrestrial prey ($\chi^2 = 5.45$, $p = 0.020$; Fig. 2b) and it was higher in allopatric than in sympatric brown trout ($\chi^2 = 13.68$, $p < 0.001$; Fig. 2b). The EPA content decreased with increasing fork length ($\chi^2 = 129.33$, $p < 0.001$) and total lipid content ($\chi^2 = 11.62$, $p < 0.001$) of individuals, and it was not affected by sex ($\chi^2 = 0.24$, $p = 0.627$; Fig. 2b). The DHA content in trout tissues decreased with increasing reliance on terrestrial prey ($\chi^2 = 14.70$, $p < 0.001$; Fig. 2c), but it did not differ between allopatric and sympatric populations ($\chi^2 = 1.71$, $p = 0.191$; Fig. 2c). DHA decreased with increasing fork length ($\chi^2 = 13.23$, $p < 0.001$) and total lipid content ($\chi^2 = 117.77$, $p < 0.001$) of individuals, and it was higher in males than females ($\chi^2 = 5.50$, $p = 0.019$; Fig. 2c).

The brain volume increased with increasing content of DHA (full dataset: $\chi^2 = 3.47$, $p = 0.063$, dataset with outlier excluded: $\chi^2 = 7.31$, $p = 0.007$; Fig. 3) and total lipids in trout tissues (full dataset: $\chi^2 = 7.82$, $p = 0.009$, dataset with outlier excluded: $\chi^2 = 3.49$, $p = 0.062$). It was larger in females than in males (full dataset: $\chi^2 = 7.67$, $p = 0.006$, dataset with outlier excluded: $\chi^2 = 6.22$, $p = 0.013$), but it did not differ between allopatric and sympatric brown trout (full dataset: $\chi^2 = 1.24$, $p = 0.266$, dataset with outlier excluded: $\chi^2 = 0.40$, $p = 0.528$). The brain volume was positively related to the fork length of individuals (full dataset: $\chi^2 = 799.2$, $p < 0.001$, dataset with outlier excluded: $\chi^2 = 897.3$, $p < 0.001$). The brain morphology (i.e. PC 1 and PC 2) was not significantly influenced by DHA or total lipids contents, sex, fork length, or competition with brook trout (see Appendix S2).

**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 resulted in lower content of n-3 LC-PUFA (i.e. EPA and DHA) in muscle tissues of brown trout in allopatric as well as in sympatric populations. The relative content of n-3 LC-PUFA also decreased with increasing fork length and total lipid content of individuals. In contrast, ALA being a
Fig. 2 Association between reliance on terrestrial prey and mean content of ALA (a), EPA (b), and DHA (c) in trout tissues. Plotted values are residuals from a LMM that included fork length and total lipid content as fixed factors and stream (factor with three levels) as a random intercept. Sample size in all three plots is $N=88$. The full black line is the regression line for the pooled dataset. The full black line is missing in (a), because there was no significant relationship for the pooled dataset.

Fig. 3 Association between the content of DHA in trout muscle tissues and brain volume. Plotted values are residuals from a LMM that included fork length and total lipid content as fixed factors and stream (factor with three levels) as a random intercept. The fitted lines are based on dataset excluding the outlier, but the outlying data point is included in the plot and it is highlighted by red colour. The full black line is the regression line for the pooled dataset.

precursor for endogenous synthesis of n-3 LC-PUFA (Pilbecky et al. 2021) has increased in larger (and presumably older, i.e. Bowker 1995; Öhlund et al. 2008) individuals and in individuals that had higher total lipid content. The higher EPA content in allopatric than in sympatric brown trout, but similar DHA content between the populations indicates that sympatric individuals were at least partially able to compensate for the reduced dietary intake of n-3 LC-PUFA.
by the endogenous synthesis and retention of these critical biomolecules (Murray et al. 2014). The content of ALA and EPA did not differ between the sexes, but females had lower DHA than males. The 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) across allopatric and sympatric populations. The relative brain volume also increased with increasing total lipids content in the muscle tissues, and it was higher in females compared to males. These findings demonstrate that intra-specific differences in diet quality in these wild fish, foraging at interface of aquatic and terrestrial food webs, are directly linked to their brain size.

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 and Wood 2005; Lund et al. 2012; Hall et al. 2014), that the availability of structural lipids, especially DHA, and not just total lipids is key for brain development in these aquatic vertebrate consumers. Our findings suggest 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 al. 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 (Kverková et al. 2022) and at the intra-specific level (Kotrschal et al. 2013). In fishes, brain size has been shown to correlate positively at the intra-specific level with learning (Marhounová et al. 2019), efficiency of antipredator responses (van der Bijl et al. 2015), or trophic position (Edmunds et al. 2016), which can have direct positive impact on fitness of wild individuals. However, large brain size is also associated with fitness costs, including reduced fecundity (Kotrschal et al. 2013), weaker immune response (Kotrschal et al. 2016), or deterioration of cognitive capacity with age (Boussard et al. 2021). Interestingly, the above-mentioned benefits of large brain appear to be higher in females than in males (e.g. Kotrschal et al. 2013; van der Bijl et al. 2015). The higher fitness benefits of the large brain and corresponding higher investments of n-3 LC-PUFA to its development might explain why female brown trout grew larger brain size than males despite having lower DHA contents in their muscle tissues. It should be also noted that while dietary supply of omega-3 LC-PUFA is determinant of 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 and 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 depend on temporal scales over which the consumption of low- and high-quality diets occurs (Brett et al. 2009; Murray et al. 2014). This is particularly important in the context of brain size, which in wild fishes has been shown to change across seasons in response to cognitive demands (McCallum et al. 2014; Versteeg et al. 2021). 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 and muscle, but no significant effect on brain size and performance in a cognitive test. In contrast, a longer study (i.e. 21 weeks of dietary treatment) by Lund et al. (2012) on juvenile pikeperch Sander lucioperca demonstrated that dietary n-3 LC-PUFA deprivation shortly after hatching can induce permanent reductions of DHA content and size of the brain, which can result in lower cognitive capacity (Lund et al. 2014). The reliance on terrestrial prey in our study was estimated based on bulk δ13C values of fin clips, which indicate dietary carbon on the temporal scale of several weeks (Jardine et al. 2005; Layman et al. 2012). A previous study has shown that the dietary shift of our focal species, brown trout, in sympathy with invasive brook trout can occur early in ontogeny and remains stable in later life stages (Cuchrousset et al. 2007; Závorka et al. 2017). 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 sympathy with the invasive species (Lovén Wallerius et al. 2017, 2022; Larranaga et al. 2019). Thus, the intra-specific dietary differences in our study likely represent a long-term specialization of individuals that might have a permanent effect on the 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 endogenous 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 habitats that are deprived of n-3 LC-PUFA. Similarly, other consumers have been shown to increase synthesis of DHA when consuming n-3 LC-PUFA deprived terrestrial
macrounvertebrates (Mathieu-Resuge et al. 2021; Twining et al. 2021b). However, our results indicated limits of this adaptation as the relative content of DHA in sympatric and allopatric trout decreased with increasing reliance on terrestrial prey. 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, 2019; Závorka et al. 2021). Our findings thus accentuate the suggestion that the trade-off between endogenous synthesis and dietary acquisition of n-3 LC-PUFA might be an important driver of phenotypic adaptations across vertebrate consumers (Twining et al. 2021a).

We also found that larger and presumably older individuals (i.e. Bowker 1995; Öhlund et al. 2008) across populations relied more on terrestrial prey and had lower EPA and DHA contents in muscles. In addition, individuals with higher total lipid content, yet lower EPA and DHA, indicate a possible dichotomy between rapidly growing early ontogenetic stages and individuals that selectively invest EPA and DHA for development of their brain (Brenna 2011; Chaguaceda et al. 2020). This age-dependent effect can be explained by the fact that 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 only been recognized under laboratory conditions (Pilecky et al. 2021). We have shown the link between n-3 LC-PUFA deprived diets and fish brain size in the context of competition with an invasive species. Ongoing global change is predicted not only to reduce n-3 LC-PUFA in primary consumers (Hixson and Arts 2016) but also possibly increase accumulation of n-3 LC-PUFA at the higher trophic levels (Heilpern et al. 2021; Pilecky et al. 2022). However, it is still poorly understood how the brain of wild fishes and other vertebrate consumers will respond to changes in dietary intake of n-3 LC-PUFA. There are in fact numerous unknown factors driving the evolution of brain size in fishes (Mitchell et al. 2020); therefore, explicit consideration of diet quality may improve our capacity to predict evolutionary and developmental responses of the brain. Our results indicate that n-3 LC-PUFA deprived diet 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 has a high potential to affect fitness of vertebrate consumers (Twining et al. 2021a; 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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Author contribution statement LZ, JH, and MJK conceived and designed the experiments. LZ and MLW performed the experiments with contribution of MJK and JH. LZ analysed the data. LZ wrote the manuscript; other authors provided editorial advice.

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Availability of data and materials Once the manuscript is accepted, all data will be archived at figshare.com (https://doi.org/10.6084/m9.figshare.14400983).

Declarations

Conflict of interest The authors have no conflict of interests to declare.

Ethics approval 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.

References

Aas Ø, Cucherousset J, Fleming IA, Welter C, Hjörsej J, Buoro M et al (2018) Salmonid stocking in five North Atlantic jurisdictions: identifying drivers and barriers to policy change. Aquat Conserv Mar Freshwat Ecosyst 28(6):1451–1464. https://doi.org/10.1002/aqc.2984
Araújo MS, Bolnick DI, Layman CA (2011) The ecological causes of individual specialisation. Ecol Lett 14(9):948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x
Arts MT, Brett MT, Kainz MJ (eds) (2009) Lipids in aquatic ecosystems. Springer Science & Business Media, Berlin
Böh m M, Schultz S, Koussoroplis AM, Kainz MJ (2014) Tissue-specific fatty acids response to different diets in common carp (Cyprinus carpio L.). PLoS ONE 9(4):e94759. https://doi.org/10.1371/journal.pone.0094759
Boogert NJ, Madden JR, Morand-Ferron J, Thornton A (2018) Measuring and understanding individual differences in cognition. Philos Trans R Soc Lond B Biol Sci 373(1756):20170280. https://doi.org/10.1098/rstb.2017.0280
Boussard A, Amcoff M, Buechel SD, Kotschal A, Kolm N (2021) The link between relative brain size and cognitive ageing in female guppies (Poecilia reticulata) artificially selected for variation in brain size. Exp Gerontol 146(111218):20170280. https://doi.org/10.1016/j.exger.2020.111218
Bowker DW (1995) Modelling the patterns of dispersion of length at age in teleost fishes. J Fish Biol 46(3):469–484
Brenna JT (2011) Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early post-natal life. Matern Child Nutr 7:59–79

Brett MT, Kainz MJ, Taipale SJ, Seshan H (2009) Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proc Natl Acad Sci 106(50):21197–21201. https://doi.org/10.1073/pnas.0904129106

Brett MT, Bunn SE, Chandra S, Galloway AW, Guo F, Kainz MJ et al (2017) How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? Freshw Biol 62(5):833–853. https://doi.org/10.1111/fwb.12909

Chaguaceda F, Eklöv P, Scharnweber K (2020) Regulation of fatty acid composition related to ontogenetic changes and niche differentiation of a common aquatic consumer. Oecologia 193:325–336. https://doi.org/10.1007/s00442-020-04668-y

Colombo SM, Wacker A, Parrish CC, Krieg F, Kaushik Ebm N, Guo F, Brett MT, Kainz MJ, Arts MT (2017) A fundamental dichotomoy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. Env Rev 25(2):163–174. https://doi.org/10.1139/er-2016-0062

Cucherousset J, Aymes JC, Santoul F, Cereghino R (2007) Stable isotope evidence of trophic interactions between introduced brook trout Salvelinus fontinalis and native brown trout Salmo trutta in a mountain stream of south-west France. J Fish Biol 71:210–223. https://doi.org/10.1111/j.1095-8649.2007.01675.x

Ebm N, Guo F, Brett MT, Bunn SE, Kainz MJ (2021) Polyunsaturated fatty acids in fish tissues more closely resemble algal than terrestrial diet sources. Hydrobiologia. https://doi.org/10.1007/s10750-020-04445-1

Edmunds NB, McCann KS, Laberge F (2016) Food web structure shapes the morphology of teleost fish brains. Brain Behav Evol 87(2):128–139. https://doi.org/10.1159/000445973

Evangelista C, Boiche A, Lecerf A, Cucherousset J (2014) Ecological opportunities and intraspecific competition alter trophic niche specialization in an opportunistic stream predator. J Anim Ecol 83(5):1025–1034. https://doi.org/10.1111/1365-2656.12208

Fauconneau B, Andre S, Chmaitilly J, Le Bail PY, Krieg F, Kaushik SJ (1997) Control of skeletal muscle fibres and adipose cells size in the flesh of rainbow trout. J Fish Biol 50(2):296–314. https://doi.org/10.1111/j.1095-8649.1997.tb01360.x

Gladyshev MI, Sushchik NN, Tolomeev AP, Dgebuadze YY (2018) Meta-analysis of factors associated with omega-3 fatty acid production in salmonids of pre-alpine habitats. Hydrobiologia 650(1):243–254. https://doi.org/10.1007/s10750-010-0266-z

Heissenberger M, Watzke J, Kainz MJ (2010) Effect of nutrition on fatty acid profiles of riverine, lacustrine, and aquaculture-raised salmonids of pre-alpine habitats. Hydrobiologia 650(1):243–254. https://doi.org/10.1007/s10750-010-0266-z

Hixson SM, Arts MT (2016) Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. Glob Change Biol 22(8):2744–2755. https://doi.org/10.1111/gcb.13295

Horká P, Sychrová O, Horký P, Svatína M, Petusek A (2017) Feeding habits of the alien brook trout Salvelinus fontinalis and the native brown trout Salmo trutta in Czech mountain streams. KMAE 418:6. https://doi.org/10.1015/kmae/2016038

Hou Z, Fuiman LA (2020) Nutritional programming in fishes: insights from mammalian studies. Rev Fish Biol Fisheries 30(1):67–92. https://doi.org/10.1007/s11160-019-09590-y

Innis SM (2007) Dietary (n-3) fatty acids and brain development. J Nutr 137(4):855–859. https://doi.org/10.1093/jn/137.4.855

Ishikawa A, Stuart YE, Bolnick DI, Kitano J (2021) Copy number variation of a fatty acid desaturase gene Fads2 associated with ecological divergence in freshwater stickleback populations. Biol Lett 17(8):2021024. https://doi.org/10.1098/rsbl.2021.0204

Ishizaki Y, Masuda R, Uematsu K, Shimizu K, Arimoto M, Takeuchi T (2001) The effect of dietary docosahexaenoic acid on schooling behaviour and brain development in larval yellowtail. J Fish Biol 58:1691–1703. https://doi.org/10.1006/jfbi.2001.0121

Jardine TD, Gray MA, McWilliam SM, Cunjak RA (2005) Stable iso-tope variability in tissues of temperate stream fishes. Trans Am Fish Soc 134(5):1103–1110. https://doi.org/10.1577/04-124.1

Kawakita E, Hashimoto M, Shido O (2006) Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. Neuroscience 139(3):991–997. https://doi.org/10.1016/j.neuroscience.2006.01.021

Kotschral A, Rogell B, Bundsen A, Svensson B, Zajitschek S, Brännström I et al (2013) Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. Curr Biol 23(2):168–171. https://doi.org/10.1016/j.cub.2012.11.058

Kotschral A, Kolm N, Penn DJ (2016) Selection for brain size impairs innate, but not adaptive immune responses. Proc R Soc B 283:20152857. https://doi.org/10.1098/rspb.2015.2857

Kverková K, Marhounová L, Polonyiová A, Kocourek M, Zhang Y, Kotrschal A, Kolm N, Penn DJ (2016) Selection for brain size impairs innate, but not adaptive immune responses. Proc R Soc B 283:20152857. https://doi.org/10.1098/rspb.2015.2857

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harisson E, Jud ZR et al (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev 87(3):545–562. https://doi.org/10.1111/j.1469-185X.2011.01169.x

Larrenaga N, Wallerius ML, Guo H, Cucherousset J, Johnson JI (2019) Invasive brook trout disrupt the diel activity and aggregation patterns of native brown trout. Can J Fish Aquat Sci 76(7):1052–1059. https://doi.org/10.1139/cjfas-2018-0110

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harisson E, Jud ZR et al (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev 87(3):545–562. https://doi.org/10.1111/j.1469-185X.2011.01169.x

Heilpern SA, DeFries R, Fiorella K, Flecker A, Sethi SA, Uriarte M, Naeem S (2021) Declining diversity of wild-caught species puts dietary nutrient supplies at risk. Sci Adv 7(22):eabf9967

Hoshino T, Suzuki M, Kozuka Y, Hojo J, Okada O, Etoh K, Kato Y, Nishigaki K, Watanabe K, Endo S, et al. (2019) Invasive brook trout disrupt the diel activity and aggregation patterns of native brown trout. Can J Fish Aquat Sci 76(7):1052–1059. https://doi.org/10.1139/cjfas-2018-0110

Lövén Wallerius M, Näslund J, Koeck B, Johnson JI (2017) Interspecific association of brown trout (Salmo trutta) with non-native brook trout (Salvelinus fontinalis) at the fry stage. Ethology 123(12):933–941. https://doi.org/10.1111/eth.12692

Lövén Wallerius M, Moran V, Závorka L, Höjjesjö J (2022) Asymmetric competition overshapes use and territory between native brown trout (Salmo trutta) and invasive brook trout (Salvelinus fontinalis). J Fish Biol 100(4):1033–1043. https://doi.org/10.1111/jfb.15010
Lund I, Skov PV, Hansen BW (2012) Dietary supplementation of essential fatty acids in larval pikeperch (Sander lucioperca): short and long term effects on stress tolerance and metabolic physiology. Comp Biochem Physiol a: Mol Integr Physiol 162(4):340–348. https://doi.org/10.1016/j.cbpa.2012.04.004

Lund I, Höglund E, Ebbesson LO, Skov PV (2014) Dietary LC-PUFA deficiency early in ontogeny induces behavioural changes in pike perch (Sander lucioperca) larvae and fry. Aquaculture 432:453–461. https://doi.org/10.1016/j.aquaculture.2014.05.039

Maklakov AA, Simpson SJ, Zajitschek F, Hall MD, Dessmann J, Cisold F et al (2008) Sex-specific fitness effects of nutrient intake on reproduction and lifespan. Curr Biol 18(14):1062–1066. https://doi.org/10.1016/j.cub.2008.06.059

Marhounová L, Kotrschal A, Kverková K, Únět N, Němec P (2019) Artificial selection on brain size leads to matching changes in overall number of neurons. Evolution 73(9):2003–2012. https://doi.org/10.1111/evo.13805

Marzetz V, Koussoroplis AM, Martin-Creuzburg D, Kainz MJ (2021) Omega-3 PUFA deficiency early in ontogeny induces behavioural changes in pike perch (Sander lucioperca) larvae and fry. Aquaculture 432:453–461. https://doi.org/10.1016/j.aquaculture.2014.05.039

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152(1):179–189. https://doi.org/10.1007/s00442-006-0630-x

Salm K, Mathieu-Resuge M, Graziano N, Dubillot E, Le Grand F, Sourdant P, Vagner M (2021) The relationship between membrane fatty acid content and mitochondrial efficiency differs within- and between-omega-3 dietary treatments. Mar Environ Res 163:105205. https://doi.org/10.1016/j.marenres.2020.105205

Sánchez-Hernández J (2020) Drivers of piscivory in a globally distributed aquatic predator (brown trout): a meta-analysis. Sci Rep 10(1):1–10. https://doi.org/10.1038/s41598-020-68207-8

Sánchez-Hernández J, Cobo F (2018) Examining the link between dietary specialization and foraging modes of stream-dwelling brown trout Salmo trutta. J Fish Biol 93(1):143–146. https://doi.org/10.1111/jfb.13672

Scharnweber K, Gadmark A (2020) Feeding specialists on fatty acid-rich prey have higher gonad weights: pay-off in Baltic perch? Ecology 118(8):e03234. https://doi.org/10.1002/ec2.3234

Scharnweber K, Chaguaceda F, Eklöv P (2021) Fatty acid accumulation in feeding types of a natural freshwater fish population. Oecologia 196:53–63. https://doi.org/10.1007/s00442-021-04913-y

Schneider CA, Rabsch WS, Elicerit KW (2021) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089

Sers B (ed) (2013) Swedish Electrofishing RegiSter – SERS. Swedish University of Agricultural Sciences (SLU), Department of Aquatic Resources. http://www.slu.se/electrofishingdatabase. [2019-11-11]

Simpson SJ, Wood NA (2005) Timing of incorporation of docosahexaenoic acid into brain and muscle phospholipids during precocial and altricial modes of avian development. Comp Biochem Physiol B: Biochem Mol Biol 141(2):147–158. https://doi.org/10.1016/j.cbpc.2005.02.009

Sundin T, Matte H (2012) Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. Physiol Biochem Zool 85:645–656. https://doi.org/10.1086/665982

Öhland G, Nordwall F, Degerman E, Eriksson T (2008) Life history and large-scale habitat use of brown trout (Salmo trutta) and brook trout (Salvelinus fontinalis)—implications for species replacement patterns. Can J Fish Aquat Sci 65(4):633–644. https://doi.org/10.1139/C08-003

Olsson K, Stenroth P, Nyström PER, Granelli W (2009) Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? Freshw Biol 54(8):1731–1740. https://doi.org/10.1111/j.1365-2427.2009.02221.x

Pilecky M, Mårtensson M, Arts MT, Kainz MJ (2021) Omega-3 PUFA profoundly affect neural, physiological, and behavioural competences—implications for systemic changes in trophic interactions. Biol Rev. https://doi.org/10.1111/brv.12747

Pilecky M, Kämmer SK, Mathieu-Resuge M, Wassenaar LI, Taipale SJ, Martin-Creuzburg D, Kainz MJ (2022) Hydrogen isotopes (δ2H) of polysaturated fatty acids track bioconversion by zooplankton. Funct Ecol 36(3):538–549. https://doi.org/10.1111/1365-2435.13981

Pollen AA, Dobberfuhl AP, Scace J, Iguilo MM, Renn SC, Shumway CA, Hofmann HA (2007) Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. Brain Behav Evol 70(1):21–39. https://doi.org/10.1159/000101067

Strand M, Aronsson M, Svensson M (2018) Classifying avian diets: estimating animal consumption on biologs using the ArtDataBank. ArtDataBanken Rapportar 21

Suganini D, Thomas R, Yalagala PC, Tai LM, Subbaiah PV (2017) Dietary docosahexaenoic acid (DHA) as lysophosphatidylcholine, but not as free acid, enriches brain DHA and improves memory in adult mice. Sci Rep 7(1):1–11. https://doi.org/10.1038/s41598-017-11766-0

Sullivan ML, Zhang Y, Bonner TH (2014) Carbon and nitrogen ratios of aquatic and terrestrial prey for freshwater fishes. J Freshw Ecol 29(2):259–266. https://doi.org/10.1080/02705060.2014.888524

Syrränen J, Korsu K, Louhi P, Paavola R, Muotka T (2011) Stream salmonids as opportunistic foragers: the importance of terrestrial invertebrates along a stream-size gradient. Can J Fish Aquat Sci 68(12):2146–2156. https://doi.org/10.1139/f2011-118
Twining CW, Brenna JT, Lawrence P, Winkler DW, Flecker AS, Hairston NG Jr (2019) Aquatic and terrestrial resources are not nutritionally reciprocal for consumers. Funct Ecol 33(10):2042–2052. https://doi.org/10.1111/1365-2435.13401

Twining CW, Bernhardt JR, Derry AM, Hudson CM, Ishikawa A, Kabeya N, Kainz MJ, Kitano J, Kowarik C, Ladd SN, Leal MC, Scharnweber K, Shipley JR, Matthews B (2021a) The evolutionary ecology of fatty-acid variation: implications for consumer adaptation and diversification. Ecol Lett. https://doi.org/10.1111/ele.13771

Twining CW, Parmar TP, Mathieu-Resuge M, Kainz MJ, Shipley JR, Martin-Creuzburg D (2021b) Use of fatty acids from aquatic prey varies with foraging strategy. Front Ecol Evol. https://doi.org/10.3389/fevo.2021.735350

van der Bijl W, Thyselius M, Kotschal A, Kolm N (2015) Brain size affects the behavioural response to predators in female guppies (Poecilia reticulata). Proc R Soc B: Biol Sci 282(1812):20151132. https://doi.org/10.1098/rspb.2015.1132

Versteeg EJ, Fernandes T, Guzzo MM, Laberge F, Middel T, Ridgway M, McMeans BC (2021) Seasonal variation of behavior and brain size in a freshwater fish. Ecol Evol 11(21):14950–14959. https://doi.org/10.1002/ece3.8179

Závorka L, Koeck B, Cucherousset J, Brijs J, Näslund J, Aldvén D et al (2017) Co-existence with non-native brook trout breaks down the integration of phenotypic traits in brown trout parr. Funct Ecol 31(8):1582–1591. https://doi.org/10.1111/1365-2435.12862

Závorka L, Crespel A, Dawson NJ, Papantheodoulou M, Killen SS, Kainz MJ (2021) Climate change induced deprivation of dietary essential fatty acids can reduce growth and mitochondrial efficiency of wild juvenile salmon. Funct Ecol. https://doi.org/10.1111/1365-2435.13860

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