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Effects of dietary fatty acids on juvenile salmon growth, biochemistry, and aerobic performance: A laboratory rearing experiment

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ABSTRACT

A three-phase experiment measured the effects of prey quality and fasting on juvenile Chinook salmon (*Oncorhynchus tshawytscha*) performance. The first phase was designed to evaluate the effect of dietary levels of two essential fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), on salmon growth. Salmon were reared for 12 weeks on three diets varying in proportions of krill (*Thysanoessa spinifera* and *Euphausia pacifica*) and northern anchovy (*Engraulis mordax*). Supplements of DHA and EPA were added to the formulated diets to achieve DHA:EPA ratios (0.6, 0.9, and 1.5) representative of naturally occurring prey. Growth rates over 12 weeks were not significantly different among diet treatments, which may be because DHA and EPA were provisioned at or above minimum requirements. Salmon maintained DHA at high levels (> 20% of total fatty acids) across all treatments and sampling periods, whereas EPA reflected dietary concentrations after 12 weeks. Fatty acids were incorporated into salmon muscle at varying rates but on average reflected diet after 1 to 2 months, similar to bulk stable isotopes of carbon and nitrogen. The second phase of the experiment was designed to evaluate fasting effects on salmon size, growth, and lipid storage over 4 weeks. Fed fish were heavier, grew faster, and had significantly more storage lipids than fasted fish. The third phase was designed to evaluate aerobic performance in fasted fish. Critical swim speeds were found to be positively related to salmon body size and storage lipids, but not prior diet quality, evidence that larger salmon with higher energy reserves may be better suited for overwinter survival due to their ability to swim faster than smaller leaner individuals. These results provide mechanistic support for the idea that body condition prior to the first ocean winter is important for juvenile salmon survival.

1. Introduction

The overall quantity of available prey is known to influence the growth, survival, and abundance of marine populations (Alverson, 1992; Mazur et al., 2007; Renkawitz et al., 2015), but aspects of prey quality may be just as important. Prey quality can vary in terms of total energy density (e.g. lipids), biochemical composition (e.g. fatty acids), or both. Lipids are naturally occurring organic compounds that are important in energy storage, cell membrane structure, and in the biosynthesis of molecules used to regulate many physiological processes (Sargent et al., 1999; Tocher, 2003; Arts et al., 2009). Fatty acids, the primary constituent of some lipid classes, are highly variable across marine prey (Budge et al., 2002; El-Sabaawi et al., 2009; Daly et al., 2010) and may also vary seasonally within a species (Iverson et al., 2002; Litz et al., 2010).

Fatty acid composition in fishes is highly affected by their diet (Saito et al., 1996; Mjaavatten et al., 1998; Copeman et al., 2013). Essential fatty acids (EFAs), such as docosahexaenoic acid (22:6n-3; DHA),

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Abbreviations: EFA, essential fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; LA, linoleic acid; ALA, α-linolenic acid; BW, body weight; PIT, passive integrated transponder; K, krill; KA, krill and anchovy; A, anchovy; FL, fork length; ANCOVA, analysis of covariance; SGR, specific growth rate; ANOVA, analyses of variance; U_{crit}, critical swimming speed; AIC, Akaike Information Criterion; SE, steryl esters; TAG, triacylglycerols; FFA, free fatty acids; ST, sterols; PL, polar lipids; TLC/FID, thin layer chromatography/flame ionization detection; FAME, fatty acid methyl esters; PUFA, polyunsaturated fatty acid; MANOVA, multivariate analysis of variance; AD, acclimation diet

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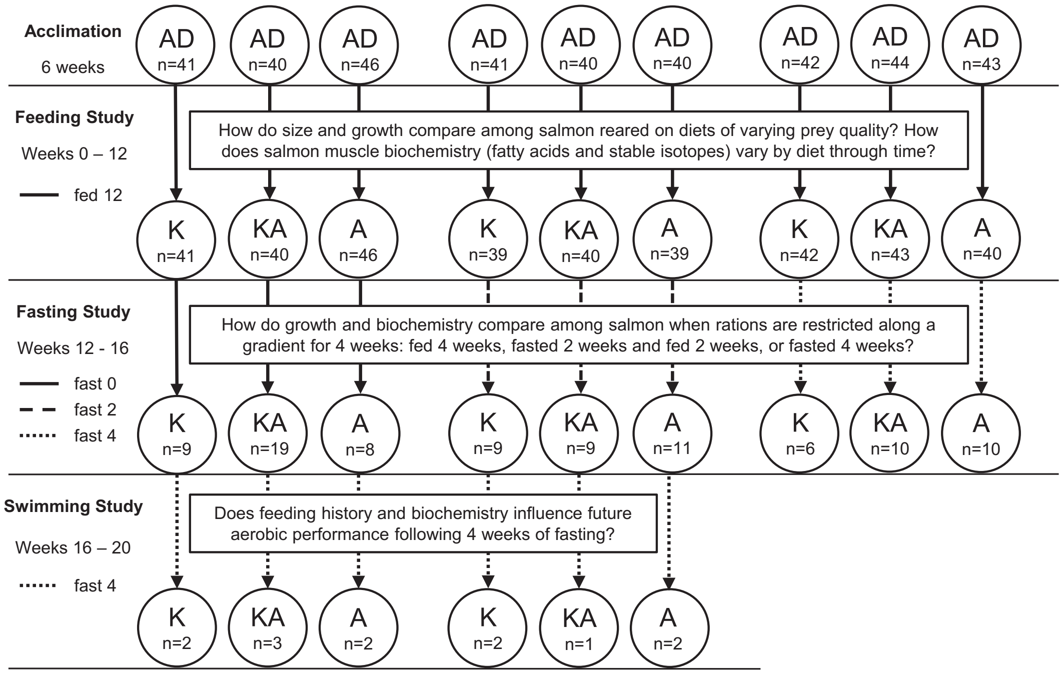

Fig. 1. Schematic overview of the 20-week experiment consisting of three phases following a 6-week acclimation period, when all fish were fed an acclimation diet (AD): (1) the feeding study, when salmon were fed either krill (K), krill and anchovy (KA), or anchovy (A) enriched diets for 12 weeks; (2) the fasting study, when fish were either fed continuously for 4 weeks, starved for 2 weeks and then fed for 2 weeks, or starved for 4 weeks; and (3) the swimming study, when fish were fasted for 4 weeks and then had their critical swimming speeds (U_{crit}, mm s^{-1}) measured. Circles indicate tanks and n = number of fish in tank.

eicosapentaenoic acid (20:5n-3; EPA), arachidonic acid (20:4n-6; ARA), linoleic acid (18:2n-6; LA), and α-linolenic acid (18:3n-3; ALA) must be obtained through diet, and insufficient amounts can lead to a suite of developmental disorders and eventual death (Sargent et al., 1995; Izquierdo, 1996; Tocher, 2010). Requirements for EFAs vary by species and life stage, yet DHA is often cited as the most important EFA during larval and juvenile phases because of its role in neural growth and development which impacts feeding efficiency, vision, behavior, and survival (Watanabe, 1993; Bell et al., 1995; Takeuchi, 2014). Ratios of DHA to EPA, which reflect relative proportions of these two functionally distinct EFAs, are commonly used to evaluate EFA requirements in larval and juvenile marine fish, with dietary ratios of DHA:EPA ≥ 2 generally considered optimal (Sargent et al., 1999; Copeman et al., 2002; Wu et al., 2003). However, ratios ≈ 1 have been shown to be sufficient in many species (Rodrìguez et al., 1997; Copeman and Laurel, 2010; Tocher, 2015).

Pacific salmon (*Oncorhynchus* spp.) migrate from freshwater to the ocean as juveniles and transition from feeding primarily on invertebrate to marine fish prey (Brodeur, 1991; Daly et al., 2009; Dale et al., 2017). Mortality during the first few months following freshwater emigration is high, and principal drivers (e.g. starvation, predation, disease) may vary regionally, temporally, and by species (Hartt, 1980; Pearcy, 1992; Beamish et al., 2004). Several studies have shown that mortality may be size-selective (Moss et al., 2005; Claiborne et al., 2011; Miller et al., 2013), and that early marine growth (Tomaro et al., 2012) and accumulation of storage lipids throughout the first summer (Beamish and Mahnken, 2001) are related to survival. Because storage lipids are easily catabolized to provide metabolic energy for growth and swimming (McCormick and Saunders, 1987; Sheridan, 1994), the amount or composition of stored lipids may be important in determining juvenile salmon survival during periods of restricted ration, which often occurs during the first ocean winter for marine fish (Schultz and Conover, 1999; Tocher, 2003; Hurst, 2007a). Higher survival of larger juveniles with greater lipid stores in fall is predicted by the “critical size, critical period” hypothesis (Beamish and Mahnken, 2001; Farley et al., 2007), which posits that smaller salmon with lower energy stores and higher metabolic rates are more likely to deplete their reserves and be more vulnerable than larger salmon during the first ocean winter.

In Northeast Pacific waters off Oregon and Washington, marine fish prey comprises a larger proportion of juvenile Chinook (*O. tshawytscha*) salmon diet through time (Brodeur, 1991; Schabetsberger et al., 2003; Daly et al., 2009). Invertebrates frequently consumed by juveniles in this region include hyperiid and gammarid amphipods, crab larvae, and krill (*Thysanoessa spinifera* and *Euphausia pacifica*); common fish prey are 0-age northern anchovy (*Engraulis mordax*; hereafter anchovy), Pacific sand lance (*Ammodytes hexapterus*), and rockfish (*Sebastes* spp.), among others (Brodeur, 1991; Daly et al., 2009; Dale et al., 2017). On average, Daly et al. (2010) found that DHA occurs in higher proportions (21% total fatty acids) in marine fish prey than invertebrate prey (14% of total fatty acids), but EPA is higher in invertebrates (29% of total fatty acids) compared to fish (21% of total fatty acids), yielding average DHA:EPA ratios that range from 0.2–0.7 for invertebrates and 0.5–1.4 for fish. Based on these measurements, it is expected that marine fish prey are more nutritionally favorable for juvenile salmon development because they are closer to the DHA:EPA ratios ≥ 1 that have been identified as beneficial for juvenile growth and survival in other species (NRC, 2011; Takeuchi, 2014).

Fatty acids, along with bulk stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), can be used as biomarkers that integrate information about a predator's diet over timescales of weeks to months (Dalsgaard et al., 2003; Fry, 2006; Parrish, 2013). The application of fatty acid biomarkers is based on the observation that fatty acids produced by primary producers (Ackman et al., 1968), or synthesized de novo by primary consumers (Lee et al., 1971), are transferred conservatively through pelagic food webs and can provide insight into trophic interactions in the marine environment. Stable isotopes are useful in that $\delta^{13}C$ varies with sources of carbon and $\delta^{15}N$ varies with nutrient source and trophic position (Fry, 2006). Integrating fatty acid and isotope biomarker data can supplement stomach content analysis and strengthen interpretation of diet data in the field, but accurate estimates of time lags between consumption and expression of biomarkers in tissues are limited. Better understanding of biomarker incorporation rates could improve interpretation of field studies of fatty acids and stable isotope ratios, especially in species that undergo ontogenetic