Changes in body composition and fatty acid profile during embryogenesis of quillback rockfish (*Sebastes maliger*)

Fletcher F. Sewall (contact author)

Cara J. Rodgveller

Email address for contact author: Fletcher.Sewall@noaa.gov

National Marine Fisheries Service, NOAA
Alaska Fisheries Science Center
Auke Bay Laboratories
Ted Stevens Marine Research Institute
17109 Pt. Lena Loop Road
Juneau, Alaska 99801

Abstract—We investigated developmental changes in the body compositions and fatty acid (FA) profiles of embryos and preparturition larvae of the quillback rockfish (*Sebastes maliger*). Comparisons of proximate composition data from early-stage embryos with data from hatched preparturition larvae taken from wild-caught gravid females indicated that embryos gain over one-third their weight in moisture while consuming 20% of their dry tissue mass for energy as they develop into larvae. Lipid contributed 60% of the energy consumed and was depleted more rapidly than protein, indicating a protein-sparing effect. Oil globule volume was strongly correlated with lipid levels, affirming its utility as an indicator of energetic status. FA profiles of early embryos differed significantly from those of hatched larvae. Differences in the relative abundances of FAs between early embryos and hatched larvae indicated different FA depletion rates during embryonic development. We conclude that some metabolically important FAs may prove useful in assessing the condition of embryos and preparturition larvae, particularly 20:4n-6, which cannot be synthesized by many marine fish and which is conserved during embryogenesis. Variability in body composition and energy use among rockfish species should be considered when interpreting any measures of condition.

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The nutritional condition of fish during their early life histories may play a major role in determining the strength of year classes because larvae must have energy stores sufficient to ensure survival to first feeding. The survival rates of early planktonic rockfish larvae may be influenced by differences in the amounts and use of endogenous protein and lipid sources during embryonic development (MacFarlane and Norton, 1999). Despite this potential importance, little is known about the biochemistry of developing rockfish embryos and larvae. Because utilization of lipid and protein may vary by species (e.g., MacFarlane and Norton, 1999) and life history stage (e.g., Norton et al., 2001), it is important to examine these variables by species at the appropriate life stage.

Quillback rockfish (*Sebastes maliger*) are a long-lived, slow-growing species of commercial importance, for which biochemical data on early life stages are lacking. Like other rockfish species of the genus *Sebastes*, they bear live young, and embryos (as post fertilization, prehatching individuals with the chorion intact) develop and hatch as larvae (individuals free of the chorion envelope) inside the maternal female before being extruded (Yamada and Kusakari, 1991). Survival during the larval phase can be vital in determining the eventual size of a rockfish cohort (Ralston and Howard, 1995). The utilization of lipids is of particular importance, as triacylglycerols (TAGs) and polar lipids (mainly phospholipids) may be the primary energy sources during rockfish embryogenesis (MacFarlane and Norton, 1999). Endogenous TAG is thought to reside mainly in an oil globule, the volume of which was identified as a main correlate of survival of black rockfish larvae (*S. melanops*) (Berkeley et al., 2004). In that study, total lipid concentration was not related to oil globule volume (OGV) or later larval survival; however, lipid levels have been correlated with survival for many other fish species (reviewed in Kamler, 1992). Research with wild-caught shortbelly rockfish (*S. jordani*) (pre-flexion larvae through juvenile stages) has indicated that the relationship of TAG to total lipids, and the usefulness of TAG as an indicator of nutritional status, depends upon life stage (Norton et al., 2001). Given this variability, it is unclear what trends may occur in total lipid levels and oil globule TAG reserves in developing quillback rockfish embryos. If OGV can be shown to be a reliable indicator of lipid levels, using this measurement would represent a substantial savings in time and cost as compared with analytical chemistry techniques.

Embryos and larvae of quillback rockfish are likely incapable of synthesizing essential fatty acids (EFAs), either entirely or at a rate which will meet their metabolic needs for growth and survival, as is the case for adults of other fish species (e.g., as reviewed

Table 1

Sample sizes for determinations of body composition and fatty acid profiles of quillback rockfish (*Sebastes maliger*) embryos (early and middle stages) and hatched, preparturition larvae (late stages). The sample unit was one maternal female, from which subsamples of embryos or larvae were obtained for use in biochemical analyses. Sample sizes varied due to inadequate subsample masses being available for some analytical procedures.

	Sample size		
Variable	Early stages (1–3)	Middle stages (4–9)	Late stages (10)
Developmental stage	5	6	4
Oil globule volume	3	4	4
Wet tissue mass	3	4	4
Moisture, protein	3	4	4
Ash	2	4	3
Lipid	3	4	4
Fatty acids	4	4	4

in Watanabe, 1982). Fish are capable of selectively catabolizing particular fatty acids (FAs) while retaining others (reviewed in Tocher, 2003). Differences in rates of individual FA use during embryogenesis would be reflected by changes in overall FA profiles as embryos develop into hatched larvae. Assessing net differences in the amounts of individual FAs present may reveal which FAs potentially contribute to variability in larval survival (e.g., due to deficiencies in particular EFAs resulting from inadequate maternal provisioning).

Our study was driven by three objectives. First, we sought to describe the amount and sources of energy consumed during quillback rockfish embryogenesis, by measuring changes in lipid and protein levels from early to late stages of development. Second, to assess the usefulness of OGV as an indicator of the energetic status of embryos and preparturition larvae, we investigated how well changes in OGV were correlated with changes in stage and biochemical composition. Last, we reduced lipids to their FA components and compared the overall FA profiles of embryos to preparturition larvae, to determine whether all FAs were used at the same rate as the total lipid during embryonic development, or whether some were used disproportionately fast while others were conserved.

Methods

Sampling

Quillback rockfish were caught 15–28 April 2006 by hook and line in southeastern Alaska on the northwest side of Chichagof Island (58°10′N, 136°21′W). Fish were caught within approximately 1 km of shore at depths of 30 to 75 m. Fifteen gravid females ranging in size from 360 to 480 mm (fork length) were transported live to Auke Bay Laboratory in Juneau, where they were kept in flow-through seawater tanks at 3.5–4°C. During a 2-week holding period, the females did not feed and did not release larvae naturally. Females were then sacrificed and a sample of embryos or larvae was manually expressed from each fish. Sample sizes available for biochemical analysis varied occasionally because each analytical procedure was destructive and required separate subsamples of embryos or larvae, and sample masses were below the minimum needed to ensure accurate analysis in some cases (Table 1). One sample of stage seven embryos was omitted from analysis due to the apparent degradation and possible resorption of embryos by the parent.

Changes in lipid and protein levels during development

Developmental stages We ranked embryos or larvae from each female in order of development (stages 1–10, from immediately after fertilization through posthatching; Fig. 1) following the descriptions of kurosoi rockfish (*S. schlegelii*) by Yamada and Kusakari (1991), and incorporating our own observations for quillback rockfish (Table 2). In quillback rockfish, we found that the retina went through many stages of pigmentation and that body pigment appeared relatively early in development and became more pronounced through time. Yamada and Kusakari (1991) include only one stage for retinal pigmentation and one for peritoneal pigment (stages 25 and 29, respectively), so we further divided the embryo stages based on these characteristics.

Developmental stages were then used for tracking changes in body composition during embryonic development. Because the durations (in days) of stages vary widely (Eldridge et al., 2002), they are not strictly appropriate for statistical analyses with linear models. In any model using developmental stage categories, the assumption that the stages represent equal intervals can distort the true patterns of change over time. Quantitative statements about rates of change in body composition ideally would be based on time since fer-

Table 2

Developmental staging scheme for quillback rockfish (*Sebastes maliger*) embryogenesis. Stages 1 through 9 represent progressively developing embryos, whereas stage-10 samples contained many hatched larvae. Equivalent stages from Yamada and Kusakari (1991) are included for comparison.

Stage	Description	Yamada and Kusakari (1991) stage
1	Embryonic shield (very small germ disc on one pole of egg)	15
2	Head fold	16
3	Optic vesicles	17
4	Optic cups, increased orbital definition	20
5	Early retinal pigmentation	25
6	Retinal pigment light, spreading throughout eye; body pigment appears as scattered dark dots along ventral side of tail	25–28
7	Very slight eye shimmer appears; body pigment increased slightly, still ventral	25–28
8	Eye shimmer increases, scattered throughout the darkening retina; body pigment increases >2×, still ventral, spots merging to form a line	25–28
9	Retina dark with a lot of shimmer scattered throughout, some black still visible; pigmentation on gut behind yolk sac and dorsally along tail	25–28
10	Dark retina covered with shimmer, body pigment blended into a dark line on ventral side of tail, spots also on dorsal side of tail and on peritoneal wall; hatched/hatching imminent; yolk not depleted	29–31

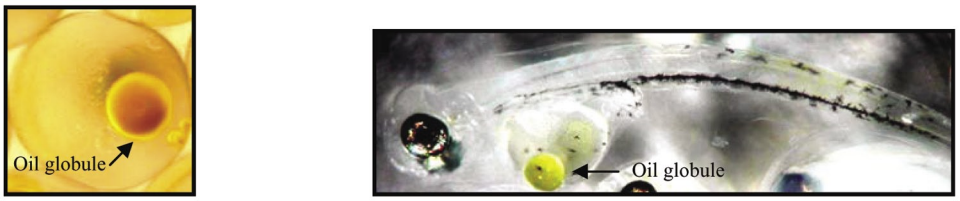

Figure 1

Quillback rockfish (*Sebastes maliger*) stage-1 embryo (left) and stage-10 hatched larva (right).

tilization; however, we did not possess data on the gestation period for quillback rockfish. The period of gestation seems to vary widely among rockfish species (e.g., 29 days for *S. flavidus* [Eldridge et al., 2002], 48 days for *S. schlegelii* [Yamada & Kusakari, 1991]), as well as the time spent at each stage of development, so we did not feel confident in assigning estimated time durations to each stage based on studies of other rockfish. However, we were more concerned with general trends during development, and net differences in body composition as embryos become larvae, than the precise rates of change among stages. In addition, other studies have reported developmental changes in body composition using stages assumed to represent equal intervals (e.g., MacFarlane and Norton, 1999); to facilitate comparisons, we also chose to follow this convention.

To assess net changes in body composition that occurred over the course of embryogenesis (i.e., differences between early embryos versus hatched, preparturition larvae), data on body compositions were averaged from three samples at the earliest available stages (stages 2 and 3) and compared with values averaged from four late-stage samples (stage 10). To describe trends and variability in lipid and protein use across all stages of development, protein and lipid masses were plotted against developmental stage and the strengths of the correlations were calculated. Samples at stages 1 and 9 were excluded from biochemical analyses due to technical constraints, such as insufficient sample masses for some processes.

Wet tissue mass The average wet tissue mass of embryos and larvae at each developmental stage was determined for use in calculations of body composition. A subsample of ~100 embryos or larvae removed from a maternal fish was placed on filter paper to drain intraovarian fluid. The subsample was then weighed to the nearest 0.1 mg, and individuals were counted under a dissecting microscope. This was repeated three times per female, and data from the three replicates were used to calculate an average wet mass per embryo or larva. These subsamples were discarded to prevent degraded or oxidized samples from being included in biochemical analyses.

The remaining embryos or larvae from a female were placed in a 20 ml glass vial capped with nitrogen and stored in a freezer at –80°C to prevent oxidation and tissue degradation prior to further processing.

Moisture, protein, and ash content A subsample of approximately 2–4 g (wet mass) of embryos or larvae, representing a composite of thousands of individuals, was used from each sample for analysis of moisture, protein, and ash (inorganic components such as phosphorous, calcium, and other minerals). To determine percent moisture, samples were placed in crucibles in a Leco Thermogravimetric Analyzer 601 (TGA 601) (Leco Corporation, St. Joseph, MI), heated to 135°C to boil off moisture, and wet and dry sample masses were compared. Percent ash was determined gravimetrically by further heating samples to 600°C to combust all organic components and weighing the remaining mass.

The dry mass percent protein was calculated as the observed nitrogen content multiplied by a factor of 6.25, based on the assumption that nitrogen accounts for 16% of the protein mass (Craig et al., 1978). Nitrogen content was determined following the Dumas method (Horwitz, 2002), using a Leco FP 528 nitrogen analyzer (Leco Corporation, St. Joseph, MI), with approximately 0.1 g dry sample mass burned at 850°C and the released nitrogen measured by thermal conductivity.

A National Institute of Standards and Technology Standard Reference Material (SRM) 1546 (pork and chicken homogenate) was used to calibrate the Leco TGA 601, and Leco calibration sample ethylenediaminetetraacetic acid (EDTA, 9.57 ± 0.04% nitrogen) was used to calibrate the Leco FP 528. Two quality assurance samples, Chinook salmon (*Oncorhynchus tshawytscha*) homogenate and walleye pollock (*Theragra chalcogramma*) homogenate, were subjected to proximate analysis along with the larval samples to verify the accuracy of protein, moisture, and ash measurements. Replicate measurements of nitrogen content were taken as a check for precision, with a target error limit of less than 15% coefficient of variation.

Carbohydrate content was not analyzed in this study because fish eggs typically have very low levels of carbohydrates, averaging 2.6% of dry mass (Kamler, 1992). Adult fish also do not typically store carbohydrates in any appreciable quantities (Brett, 1995).

Lipid content A subsample of 0.2 to 0.3 g wet mass containing hundreds of embryos or larvae was used from each of the samples for lipid analysis. Samples were processed by a modified Folch's method as described by Christie (2003). A 2:1 solution of chloroform and methanol, with 0.1 g/L butylated hydroxytoluene (BHT) to minimize oxidation, was used to extract lipids under high temperature (120°C) and pressure (1200 psi) on a Dionex ASE 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA). Extracts were washed with 0.88% KCl followed by a 1:1 (by volume) methanol/deionized water solution, both added at 25% of the extract volume, to remove co-extractables (e.g., glycerol) from the solution containing the extracted lipids. The resulting extract volume was reduced to less than 1 mL by evaporating excess solvent with a Yamato RE 540 rotary evaporator system (Yamato Scientific America, Inc., Santa Clara, CA), then drawn up by electronic pipette with sufficient chloroform to bring the volume to 1000 μL. For gravimetric analysis of total percent lipid, a 500-μL aliquot of the extract was placed in aluminum weighing pans in a fume hood overnight, allowing the solvent to evaporate and leave behind the extracted lipids. The remaining half of the extract was capped with nitrogen and stored at –80°C to minimize oxidation until further processing for FA analysis.

In quality assurance tests, the extraction method consistently yielded wet tissue lipid concentration values not exceeding 15% error compared with the certified value for Standard Reference Material 1946 (lake trout [*Salvelinus namaycush*]). Three quality control samples were also processed concurrently with the larval samples. A method blank containing no sample was used to verify that any contaminants or residues that could bias the observations of lipid mass were less than 0.01% of the average sample mass. As a check for accuracy, extraction of Pacific herring (*Clupea pallasii*) reference tissue yielded lipid concentrations that varied by less than 8% from the average value established in prior analyses. As a check for precision, one larval sample was split into two portions that yielded percent lipid values with less than 1% coefficient of variation.

Energy estimates

Total energy content, energy density, and the relative energetic contributions of protein and lipid were estimated from protein and lipid masses. Protein mass was expressed as its energy equivalent by calculating the product of protein mass and an energy density of 20.1 J/mg, and a similar calculation was made for lipids using an energy density of 36.4 J/mg—figures which are conversions of the average energy density values reported by Brett (1995). For samples having both protein and lipid analyses completed, these were combined to estimate the total energy content per individual embryo or larva, and expressed in relation to sample wet and dry masses to obtain energy density values.

Oil globule volume

Subsamples of 16 to 37 embryos or larvae (mean=24) from each female were placed in Petri dishes and photographed digitally under a dissecting microscope. Using the Clever Ruler 3.0 software (shareware published by zcstar.com), we measured two perpendicular oil globule diameters for each larva from the photos. An average oil globule volume (OGV) for each was then converted to millimeters using a stage micrometer at the same magnification. The change in OGV was determined as the difference in average OGV between early embryonic stage samples and hatched larval samples. To describe trends and variability in OGV across all stages of devel-

opment, OGV was plotted against developmental stage and the strength of the correlation calculated. The relationship between OGV and energetic status of larvae was assessed by treating lipid mass, lipid concentration, and protein mass as response variables and OGV as a predictor variable in simple linear models. Significance tests were performed with a one-way analysis of variance (ANOVA).

Fatty acid analysis

FA composition of total lipid extracts was determined by gas chromatography and mass spectrometry. To prepare lipid extracts for FA analysis, whole lipid extracts underwent acid-catalyzed transesterification to fatty acid methyl esters (FAMEs), following a procedure outlined by Christie (2003). Two mL of Hilditch reagent (0.5 N sulfuric acid [H_2SO_4] in methanol) was added to an aliquot of lipid extract which contained 0.3 mg of lipid. Before transesterification, 2050 nanograms (ng) of 19:0 FA in 50.0 μL hexane was added to each sample as an internal standard for quantification. The solution was incubated at 55°C for approximately 18 hours, and then washed with 5 mL of 5% aqueous sodium chloride (NaCl). To separate and extract the FAMEs from the aqueous solution, 4 mL of hexane was added, the solution was stirred on a vortex mixer, and the hexane layer transferred by pipette to a second container; this process was repeated with another 4 mL of hexane. Four milliliters of 2% potassium bicarbonate ($KHCO_3$) was added to the hexane containing the FAMEs to quench the esterification reaction and neutralize any remaining acid. The hexane-FAME layer was run through a sodium sulfate (Na_2SO_4) drying column to remove any residual co-extractables and water, and the resulting hexane-FAME volume reduced to approximately 1 mL in a Labconco Rapidvap (Labconco Corporation, Kansas City, MO). Prior to GC analysis, 2040 ng of 21:0 FAME in 50.0 μL hexane as an instrumental internal standard was added to each sample for use in sample recovery calculations. The FAMEs were then eluted with a temperature gradient on a Hewlett Packard 6890 gas chromatograph (Hewlett-Packard Company, Palo Alto, CA) with a 5973 mass selective detector by using a 30-m Omegawax 250 fused silica column (Sigma-Aldrich, St. Louis, MO). Five-point calibration curves were created from known concentrations of a Supelco FAME-37 standard mix (Supelco, Bellefonte, PA). Thirty of the 32 FAMEs investigated yielded calibration curves with a coefficient of determination $r^2 \geq 0.990$. As a quality assurance measure, selected calibration standards were re-injected and quantified, and the average across all FAME analytes fell within ±1.5% of the known value.

Along with the samples, quality control samples from the lipid extraction step were subjected to the transesterification procedure. Concentrations of 23 of the 28 FAMEs detected in the Standard Reference Material 1946 were within 25% of the average values obtained from six previous analyses, with none exceeding 35% error. Duplicate larval samples yielded FA concentrations with coefficients of variation less than 10% for 26 of the 29 FAs present. Six FAMEs were detected in the method blank (in order of mass: 18:0, 16:0, 22:1n-9, 17:0, 18:1n-9 *cis* and *trans*, and 14:0) and the masses of these were subtracted from the masses of those FAMEs found in each of the samples as a correction.

Statistical analysis

To determine whether FA profiles of early-stage embryos differed from hatched, preparturition larvae, raw data on FA concentrations (ng of FA per g of wet sample mass) were first converted to proportions of total FAs per sample. The relative proportions of individual FAs present in four samples of early-stage embryos were then compared to those found in four late-stage samples by analysis of similarities (ANOSIM), a nonparametric, multivariate statistical test suitable for compositional studies (Clarke and Warwick, 1994). ANOSIM was performed on a dissimilarity matrix based on the Aitchison distance (Aitchison, 1992) between all possible pairs of samples. The Aitchison distance ($D_{Aitchison}$) between two samples, A and B, is derived from the differences between the log ratios of pairs of FAs present in the two groups:

$$D_{Aitchison}(A,B) = \sqrt{\sum_{i<j}\left(\log\frac{A_i}{A_j} - \log\frac{B_i}{B_j}\right)^2}, \quad (1)$$

where j takes on values up to the number of analytes investigated—in this case, 32.

The Aitchison distance cannot be calculated in cases where the concentrations of a FA are zero in any of the samples being compared. This proved not to be a significant limitation because only one FA, 18:1n-11, was present in measurable quantities in some samples but not in others. This FA was excluded from ANOSIM analysis, but included in estimates of FA mass losses. Three other FAs (15:1n-5, 17:1n-7, and 18:2n-6 *trans*) were also excluded from analysis because they yielded zero values for all samples. We used ANOSIM to compare the ranked Aitchison distances among samples within groups and among samples between groups. This yielded the ANOSIM R statistic, which can range in value from –1 to 1, with a zero value indicating identical groups (i.e., all FAs were used at the same rate, resulting in no difference between FA compositions of embryos and hatched larvae), positive values indicating dissimilarity between groups (i.e., FAs were used at different rates, resulting in changes to the FA compositions of embryos as they developed into larvae), and negative values indicating greater dissimilarity within than between groups (i.e., a study design problem). The significance value was determined through permutations where the observed R value is compared to simulated R values assuming no difference between groups

Table 3

Quillback rockfish (*Sebastes maliger*) body composition data averaged for early-stage embryos and hatched preparturition larvae (mean ±1 SD). Comparisons of early versus late-stage samples revealed net changes that occurred during embryogenesis. "Early" included stages 2–3 embryos, and sample size (n) was 3 maternal females, except for ash (n=2). "Late" included stage-10 larvae, n=4, except ash (n=3). Comparisons only included those samples for which all proximate composition data, except ash, were available. Each sample was a composite of hundreds of embryos or larvae from the same parent. Dry masses did not sum to exactly 100% because lipid was determined by a separate process from protein and ash.

	Early	Late	% Change
Wet mass per individual (μg)	649 ±60	884 ±72	36.2
Moisture (%)	78.5 ±1.7	87.3 ±0.8	11.3
Dry mass per individual (μg)	140 ±13	112 ±15	–19.5
Protein mass per individual (μg)	90.3 ±9.9	73.0 ±12.1	–19.2
Lipid mass per individual (μg)	43.5 ±6.8	28.9 ±4.7	–33.6
Ash mass per individual (μg)	8.61 ±0.57	9.71 ±0.90	12.8
Protein (% wet mass)	13.9 ±1.08	8.22 ±0.74	–41.0
Lipid (% wet mass)	6.70 ±0.85	3.25 ±0.35	–51.4
Lipid (% dry mass)	31.0 ±1.9	25.7 ±2.2	–17.3
Total energy content per individual (J)	3.40 ±0.44	2.52 ±0.40	–25.9
Energy density (J/mg wet mass)	5.24 ±0.52	2.84 ±0.24	–45.9
Energy density (J/mg dry mass)	24.3 ±0.9	22.4 ±0.8	–7.9
Oil globule volume (nL)	27.8 ±1.2	13.7 ± .8	–50.6

(i.e., each time the ANOSIM p is calculated for a given R, its values will vary slightly).

A multidimensional scaling (MDS) plot was constructed using XLStat (Addinsoft, New York, NY) based on the Aitchison distance matrix, to illustrate the degree to which the early-stage embryonic and hatched, preparturition samples were separated based on their FA compositions. Any differences in the rates at which individual FAs were depleted during embryogenesis were expected to change the overall FA profiles over time; any net change in overall FA profile that occurred between early embryonic and later larval stage samples were revealed in the MDS plot.

In order to describe which individual FAs were responsible for the differences in overall FA profiles between early and late stage samples, we calculated the percentages of mass lost (*IML*) for each individual FA:

$$IML = \frac{\overline{m_e} - \overline{m_l}}{\overline{m_l}} \times 100\%, \quad (2)$$

where $\overline{m_e}$ = the average mass of a FA in four samples of early-stage embryos; and
$\overline{m_l}$ = the average mass of a FA in four samples of hatched larvae.

Comparison to the percentage of total lipid mass lost enabled us to describe which FAs had been depleted most rapidly, and which had been largely conserved. Because the importance of any FA in metabolism may be revealed in a combination of the rates of use and the absolute mass used (i.e., its contribution to the overall loss of lipid), we also described changes in mass of each FA between early and late stage samples and the percentage of total FA mass loss (*TML*) they accounted for:

$$TML = \frac{\overline{m_e} - \overline{m_l}}{\overline{TM_e} - \overline{TM_l}} \times 100\%, \quad (3)$$

where $\overline{TM_e}$ = the average total mass of all FAs in four samples of early-stage embryos; and
$\overline{TM_l}$ = the average total mass of all FAs in four samples of hatched larvae.

It is important to note that total lipid masses of samples were independently determined by separate processes from the FA analysis, so total lipid did not simply reflect the summed FA masses.

Results

Body composition and energy use

As they developed, quillback rockfish embryos took on water to gain size while they consumed their stored lipids and, to a lesser extent, protein as energy sources. A typical quillback rockfish embryo gained over one-third its weight in water and lost nearly 20% of its dry mass through the observed course of development, from early embryonic stages to preparturition, hatched larvae (Table 3). Dry mass loss was comprised of 54% protein and 46% lipid.

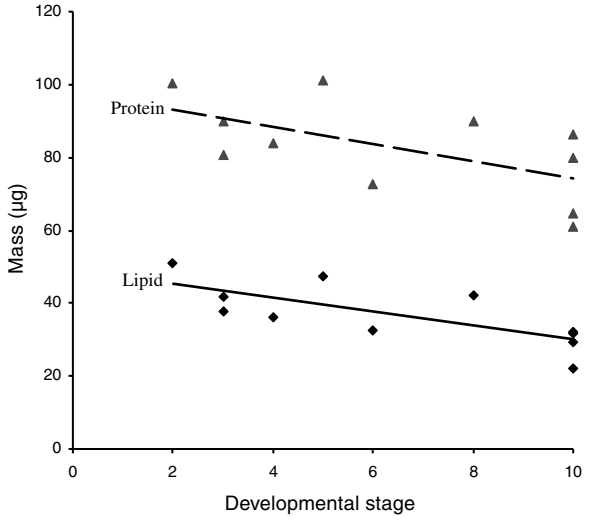

Figure 2

Protein mass (▲) and lipid mass (●) per embryo or larva by developmental stage for quillback rockfish (*Sebastes maliger*). Development progresses from left to right: Stage 2=early embryos (postfertilization); stage 10=hatched larvae (preparturition). Each point represents a single measurement of a composite sample of hundreds of embryos/larvae from one maternal female (n=11 maternal females). Only data from samples for which protein and lipid analyses were both completed are included. For lipid mass, two stage-10 points overlap and are indistinguishable. Lipid: r^2=0.54, y=–1.90x+48.96. Protein: r^2=0.35, y=–2.37x+98.02.

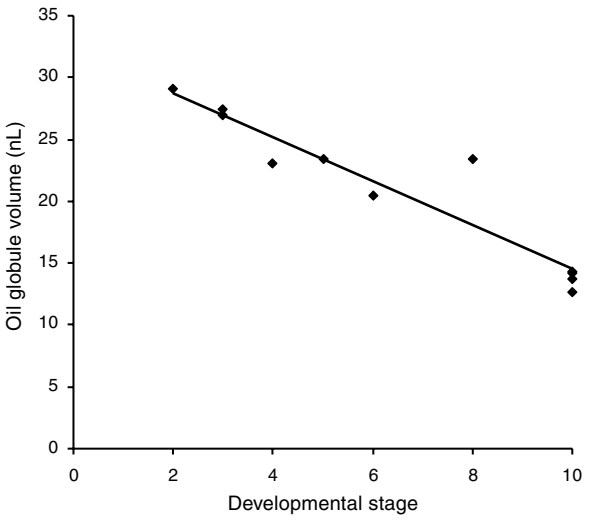

Figure 3

Oil globule volume in nanoliters (nL) by developmental stage for quillback rockfish (*Sebastes maliger*) embryos and larvae. Development progresses from left to right: Stage 2=early embryos (postfertilization); stage 10=hatched larvae (preparturition). Each point represents the mean oil globule volume calculated from diameter measurements of approximately 24 embryos or larvae from each maternal female (n=11 maternal females); two stage-10 points overlap and are indistinguishable. Only data from samples for which protein and lipid analyses were both completed are included. r^2=0.89, y=–1.78x+32.27.

While lipid and protein were both consumed in significant amounts, lipid was lost at a greater rate as a proportion of initial lipid mass (34%) than was protein (19%). Though both declined during development, there was greater variability and a weaker correlation between protein mass and developmental stage than lipid mass and stage (Fig. 2).

Using these mass losses to estimate energy use (Table 3), a developing embryo consumed a minimum of 0.88 J of energy, on average, with approximately 0.53 J (60%) coming from lipid and 0.35 J (40%) from protein. The slight decrease (8%) in the energy density of dry tissue mass was due to greater proportional losses of lipids than proteins. The 26% decline in total energy content per individual was thus more a reflection of the 20% loss in total dry mass than of the changes in proportions of lipid and protein.

Oil globule volume

The volume of the oil globule in rockfish embryos and larvae reflected both their developmental stage and body composition. OGV declined by 51% from early-stage embryos to hatched larvae. The OGV was highly correlated with developmental stage (Fig. 3). As embryos progressed through developmental stages, changes in OGV indicated trends in overall lipid and protein levels. Simple linear regression analysis indicated that total lipid was significantly dependent upon OGV (Table 4); this held true whether lipid was expressed as lipid mass per individual, or concentration (percentage of wet or dry tissue mass). Protein mass also decreased with OGV, though this relationship was weaker.

Fatty acid profiles

The proportions of fatty acids (FAs) present in quillback rockfish appeared to change during their early development, as indicated by the significantly different FA compositions of early embryos versus hatched larvae (ANOSIM R=0.677, α=0.05, n=8). An MDS plot of the samples based on their Aitchison matrix distances showed a distinct separation of the early and late-stage FA profiles (Fig. 4).

The highest percentage mass losses (> 60%) of individual FAs were found to occur among the n-11 monounsaturated fatty acids (MUFAs) 18:1n-11, 20:1n-11 and 22:1n-11; and the polyunsaturated fatty acids (PUFAs) 18:3n-3 (alpha-linolenic acid) and 20:3n-3 (eicosatrienoic acid) (Eq. 2; Fig. 5). The lowest percentage losses (<20%) occurred for the saturated fatty acid (SFA) 18:0 (stearic acid), the MUFA 24:1n-9 (nervonic acid), and

Table 4

Simple linear regression parameters relating body composition (response variables) to oil globule volume for quillback rockfish (*Sebastes maliger*) embryos and preparturition larvae. Each sample was a composite of hundreds of larvae from the same parent (n=11 maternal females). Only data from samples for which protein and lipid analyses were both completed were included.

Response variable	Slope	Intercept	r^2	ANOVA F	P
Lipid mass	1.13	0.0133	0.672	$F_{1,9}$=18.45	0.002
Lipid (% wet mass)	247	−0.101	0.943	$F_{1,9}$=150.14	<0.001
Lipid (% dry mass)	403	20.3	0.701	$F_{1,9}$=99.41	0.001
Protein mass	1.40	0.0537	0.432	$F_{1,9}$=6.85	0.028

the PUFA 20:4n-6 (arachidonic acid). No groups of FAs based on degree of saturation were apparently depleted more rapidly than others, as the percentage mass losses of SFAs, MUFAs, and PUFAs were approximately equivalent to the percentage of total FA mass loss (Fig. 5).

Some FAs showed relatively little contribution to total FA mass loss despite having large initial masses, indicating that they were conserved, particularly the SFA 18:0; and the PUFA 20:4n-6 (Eq. 3, Table 5). Meanwhile, the largest absolute mass losses were found for the SFA 16:0 (palmitic acid); the MUFA 18:1n-9 (oleic acid); and the n-3 PUFAs 22:6n-3 (docosahexaenoic acid, DHA) and 20:5n-3 (eicosapentaenoic acid, EPA), which together accounted for 71% of the total loss in FAs. Thus, there were clear differences in the contributions of different FAs to the overall lipid use.

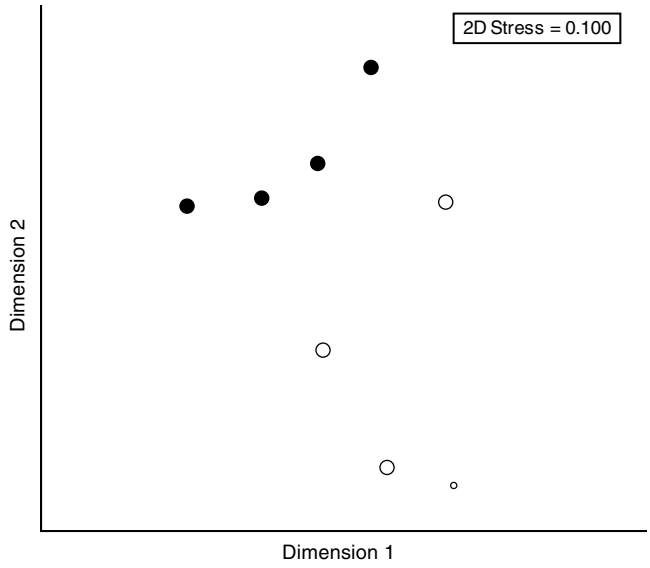

Figure 4

Multidimensional scaling plot of quillback rockfish (*Sebastes maliger*) early-stage embryos (●) and hatched, preparturition larvae (○) according to their fatty acid compositions based on an Aitchison distance matrix. Developmental stage for each sample is given in parentheses. Each sample was a composite of hundreds of larvae from the same parent (n=8 maternal females). Comparisons only included those samples for which lipid data were available.

Discussion

We found that while both lipid and protein mass are consumed by quillback rockfish embryos during development, lipid is used more rapidly and contributes a larger portion of total energy than protein. This is consistent with results from other studies of rockfish, and affirms the importance of measuring lipid levels when assessing larval condition. However, we also found differences in the specific rates of use of protein and lipid compared to other rockfish, which illustrates the diversity of patterns of energy use and changes in body composition among species.

In our study, OGV was highly correlated with lipid content. This relationship could be important for future studies researching the energetic status of rockfish embryos and preparturition larvae. Using OGV as an indicator of energy reserves at any stage of development, and knowing the relationship between OGV and developmental stage, may allow for interpreting the energetic health of embryos at any developmental stage. This is a considerable advantage for field-based studies, given the difficulty of capturing significant numbers of gravid females with embryos or larvae at the same developmental stage, and the risks of introducing experimental effects when parents are held until larvae are released. Our results also illustrate that indicators of condition applied to different species should be interpreted with differences in their biochemistries in mind (e.g., in quillback rockfish OGV is strongly related to total lipid, whereas in black rockfish the two are unrelated) (Berkeley et al., 2004).

Our study represents the first attempt to characterize FA use during embryogenesis for a rockfish species. Although aquaculture studies have investigated FA requirements for rockfish, these have typically involved manipulating the diets of adults and juveniles (e.g., Lee, 2001) and likely

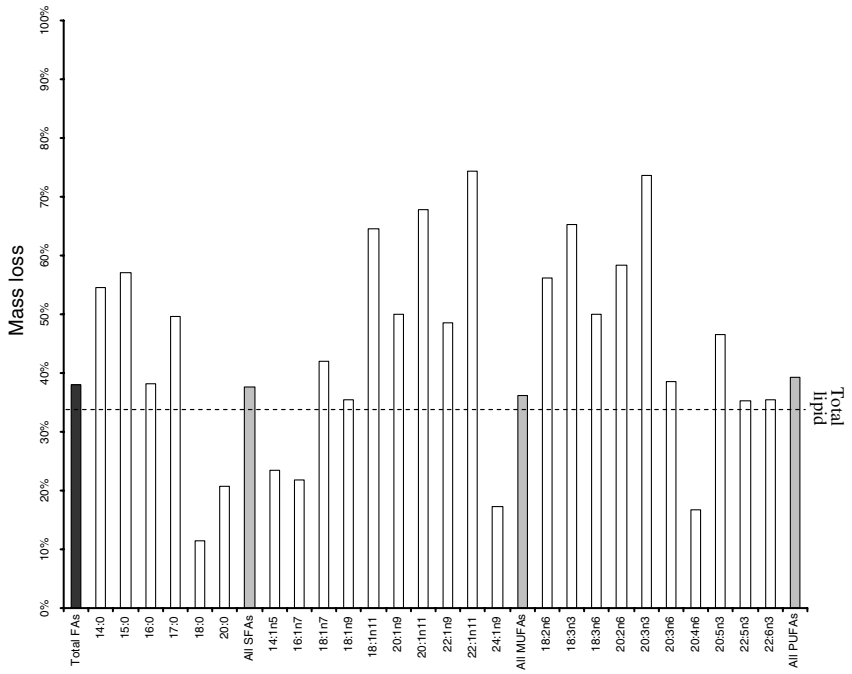

Figure 5

Percentage of individual fatty acid (FA) masses lost during embryogenesis of quillback rockfish (*Sebastes maliger*) larvae. Percentage mass losses represent differences between average FA masses per larva of four early embryonic samples and four hatched larval samples, in relation to masses present in early embryonic samples. Each sample was a composite of hundreds of larvae from the same parent (n=8 maternal females). Dashed line indicates difference in total lipid mass between embryos and larvae in relation to lipid mass in embryos.

cannot be generalized to developing rockfish embryos and larvae. Although we did not attempt to directly assess the influence of specific FAs on larval survival, our results show FAs are depleted at different rates during embryogenesis. When used in conjunction with data on total lipid levels, the relative abundances of specific conserved FAs of known metabolic importance (e.g., 20:4n-6) may be useful in assessing the condition of embryos and preparturition larvae collected from wild-caught female rockfish.

Body composition and energy use

Comparisons with other studies of rockfish revealed substantial diversity in the body compositions and energy use patterns of embryos from different *Sebastes* species—even after allowing for differences in methods and the high degree of variability in the compositional data. For example, the early stage quillback rockfish embryos studied here had lower lipid (~6.7%) and protein (~14.1%) wet tissue concentrations than those found by Eldridge et al. (2002) for late vitellogenic eggs and early embryos of yellowtail rockfish (*S. flavidus*) (~12.8% and ~21.0%, respectively). Quillback rockfish embryos had lower energy density on a dry mass basis (~24.3 kJ/g) compared with the yellowtail rockfish embryos (~27.1 kJ/g), but because of their larger dry mass, the embryos of quillback rockfish had much greater total energy per individual (3.40 J) than those of the yellowtail rockfish (~1.06 J).

The patterns of decline in lipid and protein in quillback rockfish differed somewhat from those reported by MacFarlane and Norton (1999) for yellowtail rockfish. They found that lipid as a proportion of wet mass declined 68% and protein decreased by 77%, whereas we found that lipid declined 51% and protein declined 41%. The smaller decreases in lipid and protein concentration we found may be an artifact of the different ranges of development observed (i.e., our study did not include data from unfertilized oocytes or the earliest stage-1 embryos, when protein and lipid levels were likely higher). The slightly greater decreases in protein concentration than in lipid concentration reported for yellowtail rockfish—opposite to the pattern we found with quillback rockfish—illustrates the high degree of variability among rockfish species. The results of MacFarlane and Norton (1999) for shortbelly rockfish (*S. jordani*) followed a pattern similar to ours, with lipid decreasing by 68% and protein by 55%, indicating greater conservation of protein by shortbelly rockfish

Table 5

Contributions of individual fatty acids (FAs) to total FA mass loss during quillback rockfish (*Sebastes maliger*) embryogenesis based on comparison of average FA masses for four early embryonic (stages 2–3) and four hatched larval (stage 10) samples. Each sample was a composite of hundreds of larvae from the same parent (n=8 maternal females). Results are ranked by mass loss in nanograms (ng), and grouped by degree of saturation (SFA=saturated fatty acid; MUFA=monounsaturated fatty acid; PUFA=polyunsaturated fatty acid). High variability (low precision), as indicated by coefficients of variation >10%, was found in duplicate samples for 18:1n11 (32.1%), 24:1n9 (21.2%) and 24:0 (11.3%). *=trace (≤1 ng).

Fatty acid	Mass (ng) per embryo ±1 SD	Mass (ng) per larva ±1 SD	Mass loss (ng)	% of total FA mass loss
SFA				
16:0	3250 ±481	2010 ±410	1240	13.5
14:0	660 ±111	300 ±41	360	3.9
18:0	605 ±87	536 ±119	69	0.8
15:0	113 ±9	49 ±13	64	0.7
17:0	73 ±9	37 ±6	36	0.4
20:0	11 ±1	9 ±1	2	<0.1
22:0	*	*	*	<0.1
24:0	*	*	*	<0.1
All SFAs	4710	2940	1770	19.4
MUFA				
18:1n9 *cis* and *trans*	3450 ±509	2230 ±576	1220	13.4
18:1n7	1260 ±180	731 ±148	529	5.8
16:1n7	1510 ±222	1180 ±340	330	3.6
20:1n11	269 ±100	86 ±44	183	2.0
20:1n9	255 ±42	128 ±16	127	1.4
18:1n11	115 ±35	41 ±53	74	0.8
22:1n11	80 ±36	21 ±10	59	0.7
22:1n9	24 ±5	12 ±1	12	0.1
24:1n9	64 ±9	53 ±13	11	0.1
14:1n5	10 ±2	8 ±3	2	<0.1
All MUFAs	7040	4490	2550	27.9
PUFA				
22:6n3	5950 ±959	3850 ±846	2100	23.0
20:5n3	4200 ±832	2240 ±454	1960	21.3
22:5n3	806 ±223	522 ±123	284	3.1
18:2n6	299 ±38	131 ±37	168	1.8
20:4n6	735 ±73	612 ±90	123	1.3
18:3n3	154 ±24	54 ±24	100	1.1
20:3n3	63 ±39	17 ±5	46	0.5
20:2n6	61 ±17	25 ±7	36	0.4
18:3n6	20 ±1	10 ±3	10	0.1
20:3n6	9 ±2	6 ±2	3	<0.1
22:2n6	3 ±1	*	*	<0.1
All PUFAs	12300	7470	4830	52.7

and quillback rockfish, both of which had lower initial concentrations of protein on a wet mass basis than yellowtail rockfish. From a purely energetic perspective, embryos of all three of these rockfish species show a greater decline in energy available as lipid than as protein.

The energy density of early-stage quillback rockfish embryos (5.24 J/mg) was similar to the typical value for marine spawning species of 6.0 J/mg reported by Kamler (1992). Changes in the energy density of wet tissue mass were largely a reflection of changes in the percent moisture; whereas changes in the dry tissue composition contributed less. Energy density on a dry mass basis was similar to the value for fish eggs of 23.48 J/mg reported by Wootton (1979) as an average across many species. This is not surprising, given that interspecific variation in the energy density of fish eggs is relatively low (Kamler, 1992), compared with the range of egg sizes and total energy contents.

The distinction between viviparous and ovoviviparous is a consideration in interpreting mass loss and energy data in our study because it hinges on whether the em-

bryos developing inside the mothers' bodies are supplied with maternal nutrients (viviparous), or rely entirely on their yolk sacs (ovoviviparous). Quillback rockfish have been described as viviparous (MacFarlane and Norton, 1999), and ovoviviparous (Matala et al., 2004). Previous research using radiocarbon-labeled amino acids found that embryos of black rockfish (*S. melanops*) took up nutrients from intraovarian fluid, but only at very late stages of development—presumably after they had hatched and their mouths and digestive systems were sufficiently functional (Yoklavich and Boehlert, 1991). MacFarlane and Bowers (1995) also found evidence of matrotrophy (postfertilization maternal nutrient provisioning) occurring in yellowtail rockfish because a radio-labeled phospholipid was transferred from mothers to embryos before their mouths opened, and the amount increased as they developed. Reviews of these and other studies have thus supported viviparity in rockfish (e.g., Parker et al., 2000). The reduction in dry tissue mass seen among the quillback rockfish embryos in our study was lower than the 25% to 55% range of dry mass losses typically seen in strict lecithotropes (MacFarlane and Bowers, 1995), which rely entirely on nutrients provided to the egg before fertilization, suggesting that quillback rockfish are also partly matrotrophic. The degree to which nutrition is obtained from the yolk rather than from maternal intraovarian fluids is unclear for quillback rockfish; therefore it is important to view data regarding mass loss and energy use given here as minimums.

It is likely that maternal traits (e.g., the size and age of the female parent) influence the biochemical compositions of rockfish embryos and larvae (Sogard et al., 2008). This introduces the possibility of maternal effects confounding the relationship between developmental stage and body composition (e.g., if our samples were biased towards larger females yielding the earlier stages of embryos). However, it is likely that developmental processes accounted for most of the differences that we found between early-stage embryos and hatched larvae. Developmental stage showed a much stronger relationship to lipid concentration (r^2=0.87) than did maternal length (r^2=0.39). Maternal length was only weakly correlated with developmental stage (r^2=0.26), and this correlation was largely driven by the presence of one large fish with stage 10 larvae. Removing this fish and its larvae resulted in virtually no relationship between maternal length and developmental stage (r^2=.15). This highlights one of the conclusions that can be drawn from our data: developmental stage should be accounted for when investigating maternal effects among wild caught fish with progeny at various stages.

If quillback rockfish preferentially use lipid as an energy source over protein, it would be useful to investigate how various maternal traits influence the relative rates of lipid and protein loss in embryos. For example, do embryos from older, larger, or fatter parents have greater lipid reserves, and do they exhibit lower rates of protein loss?

Why not simply use size or total energy content as indicators of viability? Such an approach is indicated by our finding that changes in total energy content per larva largely reflected changes in dry mass from early to late stages, rather than changes in the proportions of lipid and protein. In addition, there is great variability among species in the size and energy content of eggs and embryos—the early stage quillback rockfish embryos in our study were on average more than 2.6 times heavier on a dry mass basis than yellowtail rockfish embryos (Eldridge et al., 2002). Greater larval size may also confer advantages through reduced predation and increased range of feeding opportunities, and was probably the force driving the uptake of water during early development that we observed. However, various studies have found no relationship between egg size and offspring viability (reviewed in Kamler, 1992). Straightforward interpretation of the relationship of egg or embryo size and total energy content to larval viability is confounded by findings suggesting that larvae from smaller eggs often use yolk energy for growth more efficiently than those from larger eggs, and may undergo compensatory growth in later development (reviewed in Kamler, 1992). Even under conditions of food scarcity, where larger larvae may be expected to be at an advantage, results have been inconsistent; for example, larval length did not appear related to starvation resistance of black rockfish larvae (Berkeley et al., 2004).

Oil globule volume

Given the importance of lipid as an energy source for developing quillback rockfish embryos, the strong correlation of OGV with total lipid we found suggests that OGV may serve as an indicator of energetic status. Some maternal trait, such as age (e.g., Berkeley et al., 2004), may strongly influence OGV and be responsible for the variability. Investigating changes in the lipid class components (e.g., TAG and polar lipids) of the oil globules, as well as whole embryos, could provide information useful for better understanding the relationship of the oil globules to condition. The strength of the relationship between OGV and larval survival should also be investigated experimentally with quillback rockfish larvae. Using OGV as an indicator of energetic status represents a potentially large savings in resources required, compared with analytical chemistry techniques.

Fatty acid profile

The major FA components of the lipids in quillback rockfish embryos and larvae were generally similar to those reported elsewhere for many species of adult fish (reviewed in Tocher, 2003): predominantly the n-3 PUFAs 22:6n-3 and 20:5n-3; 20:4n-6 as the main n-6 PUFA; large quantities of the MUFA 18:1n-9; and 16:0 and 18:0 as the main SFAs. Previous researchers have also reported high levels of n-3 PUFAs in marine fish eggs (e.g., Tocher & Sargent, 1984); however, there can be marked interspecific differences in the precise order

of FA abundances. For example, in contrast to the quillback rockfish embryos studied here, which showed the n-3 PUFAs 22:6n-3 and 20:5n-3 in greatest abundance, Tveiten et al. (2004) reported that of 16 FAs they investigated in embryos of the spotted wolffish (*Anarhichas minor*), 18:1n-9 was predominant.

Caution must be used when attempting to apply condition indices based on FA amounts or proportions derived from other species. As lipids are broken down for use during development, the resulting FAs may be conserved as structural components of new tissues or metabolic compounds, modified into new FAs, or consumed as energy sources, and the timing and extent to which specific FAs are used varies considerably among species (reviewed in Tocher, 2003). In some marine fish, FAs appear to be utilized in a non-selective fashion (e.g., in order of their abundance) while in others, some FAs have been preferentially retained. For example, retention of 20:4n-6 was found to occur in Murray cod (*Maccullochella peelii peelii*) and trout cod (*Maccullochella macquariensis*; Gunasekera et al., 1999), Senegalese sole (*Solea senegalensis*; Mourente and Vazquez, 1996), and spotted wolffish (*Anarhichas minor*; Tveiten et al., 2004); this PUFA was also used less rapidly than the total lipid for the rockfish embryos in this study. Greater retention of 20:5n-3 has been reported in Atlantic halibut (*Hippoglossus hippoglossus*; Ronnestad et al., 1995), but this did not occur for quillback rockfish here. Tveiten et al. (2004) found that spotted wolffish embryos had lower ratios of 20:4n-6 to 20:5n-3 than those generally deemed necessary for survival in other species. In spotted wolffish embryos, the proportion of 16:0 increased, while 18:1n-9 decreased (Tveiten et al., 2004); for quillback rockfish, these FAs were used at the same rate as total FAs. This suggests that species differences must be considered in any assessment of the FA composition of developing fish.

Saturated fatty acids and monounsaturated fatty acids

The SFAs 16:0 and 18:0, and MUFAs that can be derived from them (e.g., 16:1n-7, 18:1n-9), are unlikely candidates for use as indicators of quillback rockfish nutritional or energetic status due to their relatively high abundances and the ability of all organisms to biosynthesize them. Any deficiencies in these FAs could be readily inferred from low total lipid levels.

The MUFAs with high percentage mass losses were generally present in very low amounts and likely were not of high metabolic importance. For example, the MUFA 22:1n-11, which is likely derived from calanoid copepods and transferred up through higher trophic levels in marine food chains (Saito and Kotani, 2000), was found to have the greatest rate of decrease in mass during larval development. However, its small initial mass and general absence from structural lipids in fish (Tocher, 2003) makes it likely to serve only as a minor energy source for developing quillback rockfish embryos.

Polyunsaturated fatty acids

The finding that 20:4n-6, which was the most abundant n-6 PUFA, was largely conserved seems consistent with its role as an important metabolic end product rather than a general energy source. As a precursor to the eicosanoids, a physiologically active and diverse group of hormone-like compounds, 20:4n-6 is believed to play a significant role in a variety of functions, including inducement of spawning, intercellular signaling, stress tolerance, immune response, inflammatory response, blood clotting, and is likely essential to normal growth and development (reviewed in Bell & Sargent, 2003; Tocher 2003). Several aquaculture studies have indicated that supplementing broodstock diets with 20:4n-6, within optimal concentration ranges or ratios to other FAs, results in improved egg and larval quality for a variety of marine fish species (reviewed in Bell & Sargent, 2003). Many marine fish seem to need 20:4n-6 in their diets and are unable to manufacture it from precursors (Mourente and Tocher, 1993; reviewed in Bell & Sargent, 2003); the levels of 20:4n-6 in embryos therefore likely reflect the quality of maternal provisioning. While measuring the relative abundance of 20:4n-6 may be useful in assessing condition of quillback rockfish embryos, further investigation is needed to determine what levels of 20:4n-6 may be considered deficient, and what specific effects may arise from that deficiency.

The PUFAs 20:5n-3 and 22:6n-3 were the most abundant FAs measured in quillback rockfish embryos and preparturition larvae, and decreased at approximately the same rate as total lipids. While 20:5n-3 and 22:6n-3 are important metabolic end products, they can also be consumed as major energy sources during the early life history of many marine fishes (reviewed in Tocher, 2003). Due to their abundance and role as energy sources, the amounts of 20:5n-3 and 22:6n-3 in quillback rockfish embryos are reflected in total lipid levels, and would not be informative as additional indicators of condition.

The remaining PUFAs were used more quickly than the total lipid, were generally not very abundant, and are likely of limited importance. For example, 18:3n-3, an essential fatty acid derived from marine plants, can serve as a precursor to both 20:5n-3 and 22:6n-3 in some organisms, following a metabolic pathway that is similar across widely varying taxa (reviewed in Tocher, 2003). However, the high abundances of 20:5n-3 and 22:6n-3, in combination with the relatively low levels of 18:3n-3 (<1% total FA mass), suggest that they were being synthesized from 18:3n-3 to supplement the maternally-provisioned amounts, it was only to a minor degree. Other research suggests that marine fish are largely incapable of synthesizing 20:5n-3 and 22:6n-3 from 18:3n-3 and they obtain these essential fatty acids from their diets (Tocher, 2003), in which case the embryos are likely using 18:3n-3 as a relatively small energy source. Similarly, 18:2n-6 may be of limited importance, as marine fish have limited ability to convert it to the metabolically-important PUFA 20:4n-6,

and it was present at relatively low levels in quillback rockfish embryos.

Although both lipid and protein are consumed during quillback rockfish embryogenesis, lipid is used more rapidly and contributes a greater portion of the total energy expended. Lipid is typically the most variable dry mass component of fish eggs, showing significant differences between populations and within a population over time; additionally, lipid concentration has been used as an indicator of larval viability for several species (reviewed in Kamler, 1992). Given the importance of lipid as an energy source, the strong relationship between OGV and lipid levels confirms the utility of OGV as an indicator of differences in condition of quillback rockfish embryos and preparturition larvae. Differences in FA profiles of early embryos and preparturition larvae indicate FAs are depleted at different rates during embryogenesis. More rapidly used FAs may contribute more to lipid energy use or serve as precursors in the synthesis of other FAs, while conserved FAs likely are incorporated into tissues or hormone-like compounds. The conservation of 20:4n-6, the most abundant n-6 PUFA, indicates that this essential fatty acid may well reflect the quality of maternal provisioning. The high degree of interspecific variability in body composition and energy use patterns among rockfish illustrates the need for data gathered from the species of interest, in order to make the most accurate models of energy use and most appropriate indicators of condition.

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