



Examining the utility of bulk otolith $\delta^{13}\text{C}$ to describe diet in wild-caught black rockfish *Sebastes melanops*

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ABSTRACT: Otolith carbon isotope $\delta^{13}\text{C}$ values may provide temporally resolved diet proxies in fish. If otolith $\delta^{13}\text{C}$ values reflect diet, isotope values from recent otolith and muscle tissue should correlate and known ontogenetic diet shifts should be reflected in comparisons between otolith material deposited during different life history stages. We analyzed paired otolith and muscle samples for $\delta^{13}\text{C}$ from black rockfish *Sebastes melanops* to examine the potential of otoliths to reflect diet in small (200–299 mm fork length) and large (≥ 300 mm) fish. We found a significant positive regression between $\delta^{13}\text{C}$ values from recent (~12 mo) otolith material and muscle in large fish, but not in small fish. Within individual otoliths, $\delta^{13}\text{C}$ values were enriched by ~3‰ in recent otolith edge material compared to age-0 otolith core material and were consistent with known nearshore–offshore gradients in $\delta^{13}\text{C}$ values at the base of the food web. Bulk otolith $\delta^{13}\text{C}$ appeared to provide a broad indicator of dietary carbon sources, but variation in metabolism and dissolved inorganic carbon $\delta^{13}\text{C}$ among and within individuals likely influences otolith $\delta^{13}\text{C}$ as well and limits precision. Nevertheless, the results are promising and bulk otolith $\delta^{13}\text{C}$ may be an appropriate tool to examine large trophic and ecosystem level shifts that have occurred concurrently with changes in habitat, commercial fishing, invasive species, climate change, and other direct or indirect human impacts using historic or ancient otoliths. Future studies should continue to consider the utility of bulk otolith $\delta^{13}\text{C}$ to describe diet in other marine fish using this simple approach.

KEY WORDS: Food web · Carbon isotopes · Stable isotope · Nearshore · Kelp · Micromill

INTRODUCTION

Carbon isotope ($\delta^{13}\text{C}$) values in fish otoliths may reflect trophic carbon sources and provide a complete, temporally resolved record of dietary carbon from contemporary and archived samples (Radtke et al. 1996, Elsdon et al. 2010, Nelson et al. 2011). If otolith $\delta^{13}\text{C}$ is supported as a diet proxy, archived otoliths could shed light on food web responses to past ecosystem shifts in cases where accepted soft tissue (e.g. stomachs for visual analysis or muscle for isotope analysis) diet indicators are unavailable. Otoliths are metabolically inert, continually accreting, annually

resolved, calcium carbonate (CaCO_3 ; >95%) structures in the inner ear of fishes precipitated on a small amount of protein matrix (Campana & Thorrold 2001). A major barrier to inferring dietary information from otoliths is the likelihood that otolith carbon sources include both metabolized food (diet) and dissolved inorganic carbon (DIC) from the surrounding water (Kalish 1991b, Weidman & Millner 2000, Solomon et al. 2006). Carbon from both diet and seawater are presumed to reach the otolith and surrounding endolymph fluid through the bloodstream, with DIC entering the bloodstream either across the gills or the gut during active drinking (Solomon et al. 2006).

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The proportion of otolith carbon derived from diet varies with metabolic rate and may be related to age, season, and general habitat, among other possibilities (Kalish 1991a,b, Thorrold et al. 1997, Weidman & Millner 2000, Høie et al. 2003, Solomon et al. 2006, Dufour et al. 2007, Weidel et al. 2007, Nelson et al. 2011). Despite the contribution of DIC to otoliths, a strong and consistent correlation between otolith and diet or muscle $\delta^{13}\text{C}$ values has been observed in experimental studies where diet $\delta^{13}\text{C}$ values were manipulated (Radtke et al. 1996, Nonogaki et al. 2007, Elsdon et al. 2010, Nelson et al. 2011). When both DIC and diet $\delta^{13}\text{C}$ values were manipulated simultaneously, the relationship between otolith and diet $\delta^{13}\text{C}$ values was less apparent (Solomon et al. 2006). Overall, experimental work indicates that the relationship between bulk otolith and diet $\delta^{13}\text{C}$ is dependent on the relative variation in DIC $\delta^{13}\text{C}$, diet $\delta^{13}\text{C}$, and metabolism, and that it is possible to cause bulk otolith and diet $\delta^{13}\text{C}$ to be unrelated.

The question remains whether bulk otolith $\delta^{13}\text{C}$ provides any useful indication of diet $\delta^{13}\text{C}$ in typical wild settings with natural variation in DIC $\delta^{13}\text{C}$ and metabolic rate or if more expensive alternative approaches (e.g. compound-specific isotope analysis) are necessary to extract any diet information from otoliths. This study provides an intermediate step between controlled laboratory studies and possible field applications by examining the utility of bulk otolith $\delta^{13}\text{C}$ to describe diet in wild fish where conditions cannot be controlled. This is an applied study designed to assess the utility of bulk otolith $\delta^{13}\text{C}$ to describe diet, and not an attempt to understand the mechanism behind any observed relationships, which is best accomplished in controlled experiments.

Black rockfish *Sebastes melanops* are an ideal species for examining the potential of otolith $\delta^{13}\text{C}$ values to reflect sources of dietary carbon in wild fish. First, black rockfish have large otoliths and produce wide annual growth increments during the first several years of life, providing adequate CaCO_3 material for isotopic analysis (Piner et al. 2005). Second, the natural range of DIC $\delta^{13}\text{C}$ experienced by marine fish (1.5–2.3‰; Gruber et al. 1999, Freeman 2001) is only about half of the potential range in diet $\delta^{13}\text{C}$ in nearshore food webs (3–5‰; Hobson et al. 1994, Newsome et al. 2009, Tallis 2009, Foley & Koch 2010). Large inter-individual variation in carbon isotope values among rockfish are anticipated as they are widely distributed in nearshore marine ecosystems from the Aleutian Islands, Alaska, to central California (USA) across a latitudinal $\delta^{13}\text{C}$ gradient at the base of the food web (i.e. baseline isotopic gradient,

Burton & Koch 1999, Trueman et al. 2012) and opportunistically consume a wide variety of prey (Love et al. 2002, Love 2011). In the nearshore, black rockfish feed in the water column on a wide variety of forage fish including Pacific herring *Clupea pallasii*, sand lance *Ammodytes hexapterus*, and juvenile salmon (*Oncorhynchus* spp.), and invertebrates such as crab megalopae (Love et al. 2002, Love 2011, Sturdevant et al. 2012). Third, ontogenetic shifts in diet $\delta^{13}\text{C}$ occur in black rockfish and should be reflected in otoliths. Black rockfish have one of the longest offshore pelagic life stages among rockfish, lasting 3 to 6 mo before settlement in nearshore systems (Love et al. 2002, Love 2011, Lotterhos & Markel 2012), and latitudinal movements are likely minimal given that alongshore larval dispersal is limited and adults have small home ranges (Miller & Shanks 2004, Parker et al. 2007). $\delta^{13}\text{C}$ values of nearshore food webs across the northeast Pacific Ocean are enriched by ~3–4‰ in comparison to offshore food webs, because nearshore primary production is influenced by macroalgae (e.g. kelps) that generally have higher $\delta^{13}\text{C}$ values than phytoplankton (Kline 1997, Perry et al. 1999, Miller et al. 2008). In contrast, $\delta^{13}\text{C}$ values of DIC are ~1–2‰ lower in nearshore systems compared to offshore systems of the northeast Pacific Ocean (Ortiz et al. 2000, Freeman 2001). This contrasting pattern in $\delta^{13}\text{C}$ values between organic (diet) and inorganic (DIC) sources of carbon implies that direction of $\delta^{13}\text{C}$ differences within an individual otolith could allow us to assess to what degree variation in otolith $\delta^{13}\text{C}$ is related to diet, with otolith edge enrichment following diet patterns and otolith edge depletion following DIC patterns.

We examined the relationship of $\delta^{13}\text{C}$ values between recently accreted otolith material (CaCO_3) and muscle tissue in 2 size classes and compared $\delta^{13}\text{C}$ values from otolith core to edge material in black rockfish to determine whether otoliths provide an indication of dietary carbon sources in a marine fish. This approach assumes that muscle $\delta^{13}\text{C}$ reflects diet (McCutchan et al. 2003, Perga & Gerdeaux 2005). Individuals were grouped by length, as isotopic incorporation rates scale with body size (Martínez del Rio et al. 2009) and can be as rapid as 1 mo in small fish (Weidel et al. 2011) or as long as 1 yr in larger, slower-growing fish (Hesslein et al. 1993).

MATERIALS AND METHODS

We captured rockfish in nearshore locations (depth <15 m) using hook and line sampling and trammel

nets in 2010 and 2011 from May to November along a latitudinal gradient in the northeastern Pacific Ocean from California to Alaska, USA (~35 to 60° N). Collection sites from south to north were: Big Sur (36° N, 122° W) and Monterey Bay (36° N, 122° W), California; Neah Bay, Washington (48° N, 124° W); Clayoquot Sound, British Columbia, Canada (49° N, 126° W); and Whale Bay (57° N, 135° W), Elfin Cove (58° N, 137° W), Katmai National Park and Preserve (Takli Island to Kafia Bay; 58° N, 155° W), and western Prince William Sound, Alaska (60° N, 148° W). The Whale Bay and Elfin Cove sites were sampled in 2011 and all other sites were sampled in 2010. Paired otolith and epaxial muscle samples were collected in the field from fish ranging between 201 and 396 mm fork length (FL). Fish were divided into 2 groups based on length (200–299 mm FL, $n = 20$; and 300–399 mm FL, $n = 42$) to examine the potential influence of differences in muscle isotopic incorporation rates between size classes. Growth and length at maturity curves for black rockfish indicate that fish in the large size class grow more slowly than fish in the small size class and that reproductively mature individuals occurred only in the large size class (Bobko & Berkeley 2004).

Otoliths were embedded in epoxy (Buehler EpoThin), sectioned in the transverse plane using a low speed saw, and adhered to glass slides using cyanoacrylate glue. Otoliths were examined under dissection microscopes and selected based on annual increment clarity and the absence of vaterite. Otolith material was milled from the otolith edge (recent material) and core (age-0 material) using a high-resolution micromilling system (Carpenter Microsystems). Recent otolith material from the outer edge of the otolith was defined as the last opaque and translucent band, representing up to 12 mo of growth prior to collection. Core otolith material was defined as the central opaque zone and first translucent band, spanning offshore life and settlement to nearshore systems during the first year of life. Paired core and edge material was sampled from 43 individual fish of all sizes (201–381 mm; average length \pm SD = 317 \pm 49 mm).

Epaxial muscle samples were frozen until analysis and analyzed at the University of Wyoming Stable Isotope Facility (Laramie, WY). Muscle sample C:N ratios were consistently low (mean \pm SD: 3.2 \pm 0.08), indicating a composition of nearly pure protein and a strong likelihood that lipid extraction would have a minimal influence on carbon isotope values (Sotiropoulos et al. 2004, Post et al. 2007, Ricca et al. 2007, Logan et al. 2008). Carbon isotope values ($\delta^{13}\text{C}$) from

muscle were determined using an NC 2500 Carlo-Erba or a Costech 4010 elemental analyzer coupled to a Finnegan Delta Plus XP mass spectrometer without lipid extraction. Powdered otolith CaCO_3 samples were pre-treated with a dilute NaOH solution to remove the protein matrix, and $\delta^{13}\text{C}$ values were determined using a Thermo-Finnigan GasBench interfaced with a DeltaPlus XL mass spectrometer. Isotopic results from muscle and otolith tissue are expressed as δ values, where $\delta^{13}\text{C} = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R_{sample} and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio in parts per thousand (per mil, ‰). The within-run standard deviation of $\delta^{13}\text{C}$ reference materials calibrated to internationally accepted Vienna-Pee Dee Belemnite (V-PDB) was $\leq 0.1\text{‰}$ for inorganic (CaCO_3) and $\leq 0.2\text{‰}$ for organic (muscle) samples.

$\delta^{13}\text{C}$ values from the outer edge of the otolith representing the last ~12 mo of growth and muscle samples were compared using a least squares regression for each length group. We examined residuals from each correlation to assess model fit and the possibility that collection site or year should be included in the model. $\delta^{13}\text{C}$ from otolith edge and core material from the same individuals were compared using a paired *t*-test for all fish to determine whether edge and core material reflects feeding in nearshore and offshore locations, respectively. Pairwise comparisons between otolith core and edge material assume minimal latitudinal fish movement. All analyses were conducted using R statistical software (R Development Core Team 2013).

RESULTS

Mean and standard deviations of $\delta^{13}\text{C}$ for fish collected from each site are reported for samples used to relate muscle and otolith edge $\delta^{13}\text{C}$ values (Table 1) and to compare otolith core and edge $\delta^{13}\text{C}$ values (Table 2). We found a significant positive relation between the $\delta^{13}\text{C}$ of otolith edge ($\delta^{13}\text{C}_{\text{otolith}}$) and muscle ($\delta^{13}\text{C}_{\text{muscle}}$) tissue in large fish (300–399 mm; $F_{1,40} = 49.39$, $r^2 = 0.54$, $p < 0.0001$, $n = 42$; Fig. 1). The relation was described by the linear equation $\delta^{13}\text{C}_{\text{otolith}} = 0.64(\delta^{13}\text{C}_{\text{muscle}}) + 9.13$. Carbon isotope values from otolith edge material and muscle tissue were marginally related in small fish (200–299 mm; $F_{1,18} = 3.83$, $r^2 = 0.13$, $p = 0.07$, $n = 20$; Fig. 1). Residuals followed a normal distribution for models describing the relation between muscle and otolith $\delta^{13}\text{C}$ and provided no indication of grouping by collection site or year. Otolith edge material had higher mean (\pm SD) $\delta^{13}\text{C}$ values relative to core material by an average of

Table 1. Mean \pm SD of carbon isotope ($\delta^{13}\text{C}$) values for muscle and otolith edge tissue, fork length (FL), and sample size (n) of large (300–399 mm FL) and small (200–299 mm FL) black rockfish *Sebastes melanops* collected at sites throughout the northeast Pacific Ocean; KATM: Katmai National Park and Preserve, AK; WPWS: western Prince William Sound, AK; ELFI: Elfin Cove, AK; WHAL: Whale Bay, AK; CLAY: Clayoquot Sound, British Columbia, Canada; WASH: Neah Bay, WA; MONT: Monterey Bay, CA; BIGS: Big Sur, CA

Site	Muscle $\delta^{13}\text{C}$ (‰)	Otolith edge $\delta^{13}\text{C}$ (‰)	n	FL (mm)
Large fish				
KATM	-17.5 ± 0.7	-1.9 ± 1.1	2	321 ± 16
WPWS	-17.2 ± 0.0	-2.3 ± 0.6	2	370 ± 7
ELFI	-17.7 ± 0.3	-2.1 ± 0.4	3	361 ± 34
WHAL	-17.7 ± 0.3	-2.3 ± 0.3	10	333 ± 14
CLAY	-17.0	-2.3	1	312
WASH	-16.7 ± 0.6	-1.7 ± 0.4	14	349 ± 15
MONT	-16.4 ± 0.4	-1.8 ± 0.6	2	346 ± 23
BIGS	-16.6 ± 0.4	-1.3 ± 0.5	8	334 ± 17
Small fish				
KATM	-17.2 ± 0.5	-2.3 ± 0.4	3	255 ± 15
MONT	-16.5 ± 0.4	-2.0 ± 0.5	5	250 ± 20
BIGS	-16.3 ± 0.7	-1.6 ± 0.5	12	244 ± 22

$3.1 \pm 1.1\text{‰}$ (paired t -test, $t_{42} = -17.38$, $p < 0.0001$, $n = 43$). The main assumption of the paired t -test was met, as the differences between otolith core and edge $\delta^{13}\text{C}$ were normally distributed.

DISCUSSION

Despite natural variation in metabolic rate and DIC $\delta^{13}\text{C}$ among and within otoliths, bulk otolith $\delta^{13}\text{C}$ values did provide a broad indication of dietary carbon in wild-caught black rockfish. A significant positive relation between otolith edge and muscle $\delta^{13}\text{C}$ values in large fish was consistent with variation in otolith

Table 2. Mean \pm SD of carbon isotope ($\delta^{13}\text{C}$) values from otolith core and edge material from black rockfish *Sebastes melanops* collected at sites throughout the northeast Pacific Ocean (abbreviations as in Table 1)

Site	n	Core $\delta^{13}\text{C}$ (‰)	Edge $\delta^{13}\text{C}$ (‰)	Edge enrichment (‰)
KATM	3	-5.5 ± 0.7	-1.8 ± 0.8	3.7 ± 0.4
WPWS	1	-2.9	-1.9	1.0
ELFI	1	-5.4	-2.5	2.9
WHAL	9	-6.3 ± 0.8	-2.2 ± 0.3	4.0 ± 0.8
WASH	12	-4.5 ± 1.3	-1.8 ± 0.3	2.6 ± 1.4
CLAY	1	-4.8	-2.3	2.5
MONT	6	-4.5 ± 0.7	-2.0 ± 0.5	2.6 ± 0.7
BIGS	10	-4.5 ± 0.8	-1.5 ± 0.4	3.0 ± 0.9

$\delta^{13}\text{C}$ among individuals predominantly reflecting differences in diet $\delta^{13}\text{C}$ (Fig. 1). A similar relationship in wild-caught snapper *Lutjanus ehrenbergii* was supported in the Red Sea over a much wider range ($\sim 10\text{‰}$) of diet $\delta^{13}\text{C}$ values (McMahon et al. 2011). Otolith edge $\delta^{13}\text{C}$ values were enriched in comparison to core material within individuals and were consistent with inferred patterns in baseline $\delta^{13}\text{C}$ values between offshore and nearshore food webs in the northeast Pacific Ocean. Several authors have noted that otolith $\delta^{13}\text{C}$ correlated well with diet $\delta^{13}\text{C}$ (Radtke et al. 1996, Weidman & Millner 2000, Nonogaki et al. 2007, Elsdon et al. 2010, McMahon et al. 2011) or appeared to reflect diet (Schwarcz et al. 1998, Gao & Beamish 2003, Ashford & Jones 2007, Verweij et al. 2008, Hanson et al. 2013) among other variables. While these results are consistent with diet-derived carbon driving variation in otolith $\delta^{13}\text{C}$, this observational study does not confirm the mechanism behind these patterns and we consider the possibility that our patterns reflect shifts in metabolism or DIC $\delta^{13}\text{C}$ as well.

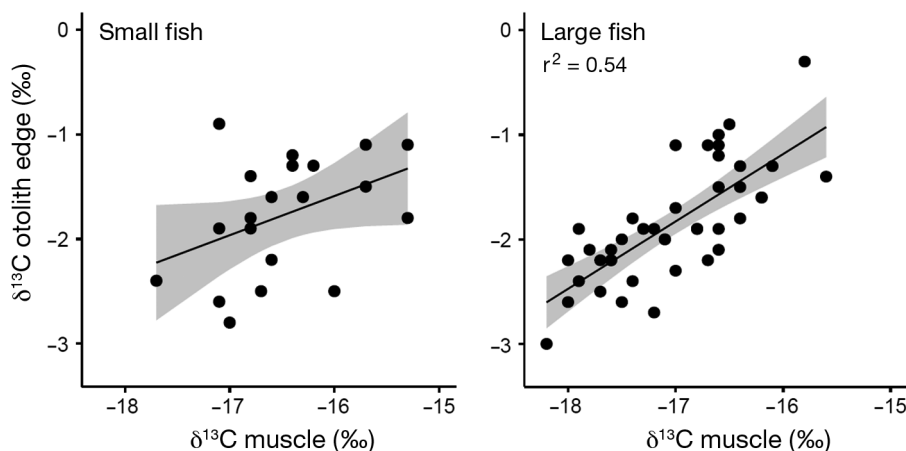


Fig. 1. Correlation between carbon isotope ($\delta^{13}\text{C}$) values in recent otolith edge material and muscle tissue for small (200–299 mm fork length, FL) and large (300–399 mm FL) wild-caught black rockfish *Sebastes melanops* from the northeast Pacific Ocean. Otolith and muscle $\delta^{13}\text{C}$ were related for large ($F_{1,40} = 49.39$, $r^2 = 0.54$, $p < 0.0001$, $n = 42$; solid line), but not small fish ($F_{1,18} = 3.83$, $r^2 = 0.13$, $p = 0.07$, $n = 20$; solid line). Shaded regions display the 95% confidence interval

Because metabolism was not measured during this study and cannot be inferred from otolith growth (Høie et al. 2003), two basic assumptions will have to be made to consider how metabolic rate may vary among and within individuals: (1) fish collected from southern locations with warmer temperatures had higher metabolic rates than fish collected in more northern locations and (2) young fish have higher metabolic rates than older fish. Both an increase in metabolic rate with temperature and a decrease in metabolic rate with age are well supported for fish (Gillooly et al. 2001, Brown et al. 2004). If variation in otolith $\delta^{13}\text{C}$ among individuals primarily reflects differences in metabolism, then individuals from southern sites would be expected to have a higher proportion of dietary carbon in their otoliths (M) and therefore lower otolith $\delta^{13}\text{C}$ compared to individuals from northern sites. Indeed, increased metabolic rates, achieved via increased temperature, have been linked to reductions in otolith $\delta^{13}\text{C}$ (Høie et al. 2003, 2004). However, the differences in otolith $\delta^{13}\text{C}$ followed an opposite pattern, with individuals from southern sites having higher otolith $\delta^{13}\text{C}$ (Table 1). Additionally, the relation between muscle and otolith $\delta^{13}\text{C}$ should be stronger in smaller, faster-growing fish, which presumably derive more otolith carbon from diet. On the contrary, stronger muscle to otolith $\delta^{13}\text{C}$ relationships were found for older fish. Within-otolith increases in $\delta^{13}\text{C}$ from core to edge material has been observed in previous studies and can be attributed to age-related shifts in metabolism (Weidman & Millner 2000, Høie et al. 2003, Dufour et al. 2007, Hanson et al. 2013). Decreasing size-specific metabolic and somatic growth rates with age are associated with declining M and an increasing use of inorganic carbon from DIC with high $\delta^{13}\text{C}$ values relative to diet. Still, shifts in metabolism only explain the within-otolith difference in $\delta^{13}\text{C}$ and not among-otolith variation in $\delta^{13}\text{C}$.

If DIC $\delta^{13}\text{C}$ was the primary driver of otolith $\delta^{13}\text{C}$ values, recent otolith edge material from nearshore residency should be depleted compared to core material from offshore life, but there were no instances where edge material was depleted compared to core material. The direction of DIC $\delta^{13}\text{C}$ gradients is likely to be consistent at each of our sampling locations due to underlying mechanisms. DIC $\delta^{13}\text{C}$ values from nearshore areas are depleted by at least 1–2‰ relative to offshore zones due to the influence of terrestrial run-off and upwelling (Ortiz et al. 2000, Freeman 2001). The magnitude of DIC $\delta^{13}\text{C}$ gradients from offshore to nearshore can increase with upwelling intensity and freshwater discharge (Simenstad et al. 1993, Nelson et al. 2011). For instance, DIC

$\delta^{13}\text{C}$ has a 4‰ range in the coastal freshwater-influenced Florida Keys (Patterson & Walter 1994) and a 12‰ range in a North Sea estuary (Gillikin et al. 2006). Even in an extreme case with large variation in DIC $\delta^{13}\text{C}$, nearshore DIC $\delta^{13}\text{C}$ values would not be higher than offshore values, as upwelled and freshwater sources of DIC both have lower $\delta^{13}\text{C}$ values than offshore surface marine DIC (Simenstad et al. 1993, Nelson et al. 2011, McMahon et al. 2013).

Our results are most consistent with among- and within-individual variation in otolith $\delta^{13}\text{C}$ predominantly reflecting diet. Among large fish, variation across individual otolith $\delta^{13}\text{C}$ was consistent with differences in diet among individuals, represented by muscle $\delta^{13}\text{C}$, an established diet proxy (McCutchan et al. 2003, Perga & Gerdeaux 2005). Offshore to nearshore differences in diet can explain within-otolith increases in $\delta^{13}\text{C}$. Primary producer $\delta^{13}\text{C}$ values are enriched in nearshore versus offshore ecosystems because of enhanced phytoplankton production and presence of macroalgae (kelp and seagrass) in nearshore areas of the northeast Pacific Ocean and elsewhere (Kaehler et al. 2000, Clementz & Koch 2001, Graham et al. 2010). Nearshore residents reflect the pattern in primary producers with a 3–4‰ increase in $\delta^{13}\text{C}$ from zooplankton (Kline 1997, Perry et al. 1999) to top marine consumers (pinnipeds and seabirds) that forage in nearshore versus offshore habitats (Hobson et al. 1994, Burton & Koch 1999). Indeed, values of black rockfish muscle $\delta^{13}\text{C}$ in this study were enriched by ~4‰ compared to published values from unidentified juvenile pelagic rockfish sampled farther offshore in the northern California Current (~41–44° N; mean -21.2‰; Miller et al. 2008).

If otolith $\delta^{13}\text{C}$ variation is related to diet, then the weaker muscle–otolith relationships in smaller individuals could be attributed to differences in the isotopic turnover in muscle tissue, with larger fish providing a better temporal match between tissues or sample size. The incremental growth and the metabolically inert properties of otoliths allow for the sampling of otolith material at annual resolution (Campana & Thorrold 2001); however, the isotopic incorporation rate of muscle tissue likely varied with fish size and growth rate. Due to metabolic demands associated with growth, smaller fish are generally known to have faster isotopic turnover rates that can be far less than 1 yr (Hesslein et al. 1993, Perga & Gerdeaux 2005, Weidel et al. 2011) that could account for the lack of correlation between $\delta^{13}\text{C}$ in recent otolith material and muscle observed in small (200–299 mm) black rockfish. The size range of small black rockfish corresponds to a size of more rapid

growth than the size range of large fish (Bobko & Berkeley 2004). For large fish >300 mm, isotopic incorporation rates of muscle tissue are likely closer to the time period represented by an annual growth increment in an otolith, making the ecological information recorded in otolith and muscle more comparable (Hesslein et al. 1993, Perga & Gerdeaux 2005, Miller 2006). Additionally, the sample size of small fish was only about half the sample size of large fish ($n = 20$ and $n = 42$, respectively), and this discrepancy could cause the strength of the relationships to differ.

Our results provide a realistic expectation of how well otolith $\delta^{13}\text{C}$ is related to diet under natural conditions. Variation in DIC $\delta^{13}\text{C}$ and metabolism likely cause some smaller differences among and within otolith $\delta^{13}\text{C}$, which may limit the precision of bulk otolith $\delta^{13}\text{C}$ and could be responsible for the scatter in muscle–otolith relationships. Lipids were not extracted from muscle samples prior to analysis, but are unlikely to be a source of variation among individuals as judged by the low variation in C:N ratios ($SD = 0.08$; Sotiropoulos et al. 2004, Post et al. 2007, Logan et al. 2008). If lipid composition is similar among individuals, lipid extraction probably would have resulted in a modest (<1%) increase in muscle $\delta^{13}\text{C}$ for all individuals (Logan et al. 2008), shifting the intercept in our regression of otolith–muscle $\delta^{13}\text{C}$ and otherwise causing little change to the regression.

The observation that variation in otolith $\delta^{13}\text{C}$ reflects diet is not necessarily at odds with experimental studies that consistently conclude that otolith carbon is principally derived from DIC (Kalish 1991b, Weidman & Millner 2000, Høie et al. 2003, Solomon et al. 2006). Instead, interpretation of otolith $\delta^{13}\text{C}$ is likely context dependent, with the relative degree of variation in the $\delta^{13}\text{C}$ of DIC and dietary sources of carbon influencing the interpretation of otolith $\delta^{13}\text{C}$ variation. Among observational and experimental studies on a wide variety of species from diverse habitats that detected a relationship between diet and otolith $\delta^{13}\text{C}$, the range of DIC $\delta^{13}\text{C}$ values was small or DIC $\delta^{13}\text{C}$ was constant (Radtke et al. 1996, Nonogaki et al. 2007, Elsdon et al. 2010, McMahon et al. 2011). When DIC and diet $\delta^{13}\text{C}$ values both varied widely due to experimentally spiked water and food treatments, otolith $\delta^{13}\text{C}$ largely reflected DIC $\delta^{13}\text{C}$ and not diet (Solomon et al. 2006). Yet, if only the trials using natural water treatments with small DIC $\delta^{13}\text{C}$ variation are examined, otolith $\delta^{13}\text{C}$ appears to track diet $\delta^{13}\text{C}$ (see well treatments in Solomon et al. 2006). A similar situation is apparent in aquatic bivalve shells, where variation in shell $\delta^{13}\text{C}$ may reflect diet when DIC $\delta^{13}\text{C}$ is fairly consistent (Poullain et al.

2010), despite shell carbon being predominantly derived from DIC (McConnaughey & Gillikin 2008). Therefore, bulk otolith $\delta^{13}\text{C}$ utility may be limited to individuals living in environments with fairly constant DIC $\delta^{13}\text{C}$. Large variation in DIC $\delta^{13}\text{C}$ naturally occurs in freshwater systems (–15 to 0‰) (Boutton 1991) and could mask diet $\delta^{13}\text{C}$ variation in otoliths of freshwater fish or anadromous fish that move across strong gradients in DIC $\delta^{13}\text{C}$.

The simple and efficient approach used in this study to assess whether otolith $\delta^{13}\text{C}$ reflects dietary inputs can be applied in other species. Continued experimental and observational studies from a variety of species and conditions are still needed to evaluate the consistency and limitations of otolith $\delta^{13}\text{C}$ values as diet proxies. If studies continue to support the use of otoliths as diet proxies, this approach could be used to examine within- and among-individual differences in dietary niche partitioning and ontogenetic variation in marine fish communities. In addition, contemporary and historic carbon sources could be compared using otoliths archived in museums and archaeological sites, which may characterize trophic or ecosystem level shifts (e.g. presence/absence of kelp) that have occurred concurrently with exploitation of keystone predators (e.g. sea otters), commercial fishing, invasive species, climate change, and other direct or indirect human impacts. For questions aimed at understanding large shifts in carbon sources of marine fish, bulk otolith $\delta^{13}\text{C}$ will likely be informative, but in other cases, an analysis of only the protein matrix (Grønkjær et al. 2013) or compound-specific $\delta^{13}\text{C}$ from dietary amino acids (McMahon et al. 2011) will be required to reconstruct dietary carbon from otoliths.

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