

Applications of Stable Isotope Analysis to Tracing Nutrient Sources to Hawaiian Gobioid Fishes and Other Stream Organisms

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Abstract

The measurement of naturally occurring stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$, measured as $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) can be used to distinguish sources of nutrients to freshwater stream fishes and other organisms in cases where such sources are isotopically distinct. We used $\delta^{13}\text{C}$ measurements of fish muscle tissue to quantify relative contributions of allochthonous (leaf litter) and autochthonous (algae) nutrients to adult gobioid fishes at Hakalau Stream, Hawaii, 1996–2002. We also used $\delta^{15}\text{N}$ measurements to delineate trophic interactions among stream organisms. We detected a strong reliance on benthic algae vs. leaf litter. The Hawaiian gobioid fishes are amphidromous and have a marine larval phase. Newly arriving larval recruits into Hakalau stream were examined isotopically to investigate if this approach could be used to quantify their relative dependence on marine and freshwater nutrient contributions. On the basis of a three-source, two isotope mixing model, the stable isotope ratios of these recruits strongly resembled those of the freshwater algal-based food web and suggest that larvae spend sufficient time in the nutrient-rich plume to register ingestion of stream-borne nutrients. However, further studies are now needed to contrast our stable isotope values of larval gobioids with known completely marine pelagic larvae.

Introduction

Fundamental to an understanding of the ecology of freshwater Hawaiian streams is the establishment of the relative importance of various sources of nutrients to higher trophic-level organisms. Within food webs supporting fishes, it is also important to delineate trophic interactions among organisms in order to understand community composition, competition, and carrying capacity. Previously, these questions were difficult to answer due to limitations inherent in conventional approaches to dietary analyses of most taxa. Recently developed isotopic tracing techniques provide the means of identifying recent nutrient sources for consumers (Lajtha & Michener, 1994; Kelly, 2000), and permit inferences about the spatial and temporal distribution of organisms which move between isotopically distinct habitats (Hobson, 1999). In both terrestrial as well as marine and freshwater systems, stable-carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) differ according to a variety of biogeochemical processes. However, in general, marine food webs tend to be more enriched in ^{13}C compared to freshwater and terrestrial C-3 food webs (Chisholm *et al.*, 1982; Hobson & Sealy, 1991; Hobson *et al.*, 2000). This phenomenon is related to the fact that heavier (i.e., those containing a ^{13}C vs. a ^{12}C atom) atmospheric CO_2 molecules tend to be dissolved into marine waters as bicarbonate ions and leave atmospheric sources of CO_2 more depleted. Further, within marine systems, inshore or benthically linked food webs tend to be more enriched in ^{13}C than pelagic food webs (Hobson & Welch, 1992; France, 1995). Within freshwater or marine systems, algal-based food webs also tend to be enriched in ^{13}C compared to phytoplankton-based food webs. Stable-nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$) in marine food



Figure 1. Overview of the mouth of our study site, Hakalau Stream, Hawai‘i. Photograph courtesy of Dr. Anne Hansen (University of Colorado).

webs also tend to be more enriched than in terrestrial or freshwater food webs (Michener & Schell, 1994; Hobson *et al.*, 1999). However, ^{15}N in consumer tissues shows a stepwise increase with trophic level, and $\delta^{15}\text{N}$ measurements have been used successfully to delineate trophic relationships among organisms, especially within marine systems (Hobson & Welch, 1992; Michener & Schell, 1994). The use of at least a dual-isotope approach in food web studies can be an effective tool in clarifying spatial and temporal aspects of the feeding history of organisms (Lajtha & Michener, 1994).

We examined stable isotope patterns in 5 species of native Hawaiian gobioid amphidromous fish and their supporting food web. These included *Lentipes concolor*, *Sicyopterus stimpsoni*, *Stenogobius hawaiiensis*, and *Awaous guamensis*, members of the Family Gobiidae and a fifth species from the Family Eleotridae, *Eleotris sandwicensis*. Previously, Sorensen and Hobson (in press) presented results of isotopic analyses of a similar dataset aimed at examining specifically the question of dietary histories of feeding in new larval recruits. Here, we were interested more in identifying sources of nutrients to freshwater adults with particular emphasis on evaluating the role of (allochthonous) leaf litter and (autochthonous) epiphytic algae. In addition, we examined newly arriving larval recruits from marine habitats to see if there was evidence of use of freshwater-derived nutrients from the river plume prior to upstream migration using newly developed isotope mixing models. Although few Hawaiian streams have well-developed estuaries, observations suggest that fresh water might exert a considerable influence on the coastal environment because large amounts of colored organic matter can be seen hundreds of meters from river mouths much of the year. The contrast can be dramatic because the ocean is nutrient-poor and clear. Finally, we surveyed a number of representative inshore and offshore marine species to examine the range of isotopic variation potentially influencing amphidromous species during their marine phase.

Methods

Our study was conducted at Hakalau Stream (Island of Hawai'i, $19^{\circ}53'55.5''\text{N}$, $155^{\circ}07'32.0''\text{W}$; photograph Fig. 1; map in Schoenfuss *et al.*, 1997). Hakalau Stream is relatively typical of many Hawaiian streams. It is of moderate size, and, while it has been impacted by agriculture, there has been little development and it continues to attract large numbers of all native amphidromous species. Accordingly, it has also been the subject of much study (e.g., Tate, 1997; Nishimoto & Kuamo'o, 1997; Radtke *et al.*, 2001). Stream flow in Hakalau Stream is highly variable, and while it is not gauged, gauged flows in nearby Honoli'i Stream ($19^{\circ}46'00''\text{N}$, $155^{\circ}09'16''\text{W}$) which is similar in size suggest it likely fluctuates between approximately $50\text{ m}^3\text{ sec}^{-1}$ in early spring to about 1% of this value in the summer. We sampled and analyzed juvenile and adult gobies and possible nutritional sources for them within Hakalau Stream between 1996–2002 for one month during peak (February–April; Nishimoto & Kuamo'o, 1997) migration each year. On several years sampling was further restricted because of flooding and drought, and in 2000 and 2001 no samples could be obtained. For three years, we also examined nutrient particulate organic matter (POM) at inshore and offshore marine locations.

For one month each spring, amphidromous migratory post-larvae were individually collected by researchers equipped with snorkels and small hand nets at four locations within Hakalau Stream and identified by using the key of Tate *et al.* (1992). Sampling locations included the freshwater interface where Hakalau Stream empties directly into the Pacific Ocean, approximately 100 m upstream from the freshwater/marine interface, and a riffle located above the bridge where adults of two species (*A. guamensis* and *S. stimpsoni*) were found in abundance (adults are not often found below this location). Another site was located approximately 2 km above several terminal waterfalls (300 m above sea level) where only adult *L. concolor* were found ($19^{\circ}52'38.3''\text{N}$, $155^{\circ}09'15.1''\text{W}$). Each year we attempted to collect at least 5 adults of each species at the upstream sites as well as at least 10 new recruits from both the river mouth and bridge sites. New recruits were frozen within 8 h of capture, while adults were euthanized and a 1 g sample of caudal muscle removed and frozen.

To assess possible nutrient sources for juvenile and adult gobies, we sampled in both coastal, offshore marine, and freshwater environments. Because the relative roles of allochthonous and autochthonous production has not been assessed in Hawaiian freshwater streams which tend to contain large amounts of leaf litter and other detritus as well as freshwater algae (which covers the rocks), we sampled both. Reports that adult gobies consume a combination of algae, stream invertebrates, and detritus (Kido, 1996, 1997; Way & Burky, 1991) further emphasized that both detritus and algae might be important sources of nutrients. Both allochthonous (leaf litter and associated detritus floating in the water and accumulating on the bottom) and autochthonous material (epiphytic algae growing on rocks) were sampled in the lower regions of Hakalau by handnet in 1999 and 2002. We obtained several samples of POM from nearby coastal regions by trawling a 0.5-m plankton mesh net (333- μ mesh) from a small boat at a distance of 0.5–1.0 km offshore within 5 m of the surface in 1999, 2000, and 2002. In all cases, samples were taken a few km to the southeast and seemingly out of the immediate influence of freshwater (salinities were in excess of 35‰). Another set of oceanic POM samples was taken by an ocean-going research ship using a 0.5 m 333- μ plankton net and towing for three 30-min intervals in the top 10 m approximately 10 km offshore of Hakalau Stream (approximately 19°10'N, 156°45'W) in 2002. All POM samples contained zooplankton the larger of which were removed under a microscope. In addition to these samples, some reference organisms (fishes and molluscs) were also collected from both Hakalau Stream and offshore fisheries.

For analysis, samples were freeze-dried, powdered, and lipids removed by using a 2:1 chloroform:methanol solution. For POM, we treated samples with 1N HCl to remove carbonates. Leaf litter and stream algae were processed by sorting and cleaning in distilled H₂O, dried at 60 °C, and powdered in an analytical mill. For final analysis, residual solvents were removed from processed samples by drying in an oven (60 °C), and then 1 mg of each powdered homogenized tissue was loaded into a tin cup and combusted at 1200 °C in a Europa ANCA-GST elemental analyzer (Europa Scientific Ltd., Crewe, UK). We analyzed the resultant gas with a Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS; Europa Scientific Ltd., Crewe, UK) with every 5 unknowns separated by 2 laboratory standards. The isotopic composition of tissues is reported in δ notation as parts per thousand according to

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$$

where X is ¹³C, or ¹⁵N, and R is the corresponding ratio ¹³C/¹²C or ¹⁵N/¹⁴N. The standard for carbon was the Pee Dee Belemnite and for nitrogen atmospheric air (AIR). Replicate measurements of internal laboratory standards indicate measurement errors of 0.1‰ for carbon and 0.3‰ for nitrogen isotope measurements.

Results

Stream-resident adult fish and primary production

With the possible exception of 1998, adult *A. guamensis* and *L. concolor* fishes had very similar isotope ratios (Fig. 2). Nevertheless, slight shifts in isotopic values were apparent between years for all three species, and an overall ANOVA measured a significant effect of species ($F_{3,56} = 10.68$, $p < 0.001$) and year ($F_{3,56} = 3.18$, $p = 0.033$) on adult goby $\delta^{13}\text{C}$ values. There was no interaction between species and year ($F_{3,56} = 2.10$, $p = 0.113$). *Sicyopterus stimpsoni* was slightly more enriched in ¹³C compared with the other 3 species which did not differ among themselves (Tukey's, $p > 0.05$). Accounting for species differences, the years 1997 and 1998 were more enriched in ¹³C than the years 1999 and 2002 ($p < 0.05$), each of which represented a homogenous subset.

We found a small but significant effect of species ($F_{3,56} = 97.48$, $p < 0.001$) and year ($F_{3,56} = 21.56$, $p < 0.001$) on adult goby $\delta^{15}\text{N}$ values (Table 1). No interaction between species and year was detected for adult goby $\delta^{15}\text{N}$ values ($F_{3,56} = 0.86$, $p = 0.435$). All species formed homogenous subsets in the following order of lowest to highest mean $\delta^{15}\text{N}$ values: *S. stimpsoni*, *A. guamensis*, *L. concolor*, and *E. sandwicensis*. Accounting for species differences, the years 1998 and 2002 formed one homogenous subset for $\delta^{15}\text{N}$ values, and the years 1997 and 1999 two other homogenous subsets, respectively.

Stable isotope values of epiphytic algae did not differ among years. However, leaf litter differed slightly but significantly in $\delta^{15}\text{N}$ values between the two years of sampling, 1999 and 2002 ($t = 3.85$, $p = 0.004$). This was also the case for $\delta^{13}\text{C}$ values ($t = 2.21$, $p = 0.03$). Leaf litter was significantly less enriched in ^{13}C than algae ($t > 8.1$, $p < 0.001$, for all comparisons) (Table 2).

For marine POM, we found no effect of year on either $\delta^{15}\text{N}$ ($t = 1.49$, $p = 0.21$) or $\delta^{13}\text{C}$ ($t = 1.03$, $p = 0.41$) values of inshore POM (Table 2). Offshore POM had somewhat lower $\delta^{15}\text{N}$ values compared with the combined (2002 and 1999) inshore values ($t = 4.08$, $p = 0.03$). No difference was found for inshore and offshore marine POM $\delta^{13}\text{C}$ values ($t = 0.36$, $p = 0.73$).

New recruits

We found a high level of similarity between the isotope values of all gobioid fish, with all mean annual values falling between 6.1 and 7.1‰ for $\delta^{15}\text{N}$, and -19.0 and -17.3‰ for $\delta^{13}\text{C}$ values (Table 3). There was a significant effect of year ($F_{4,237} = 16.21$, $p < 0.001$) and species ($F_{2,237} = 6.37$, $p = 0.002$) on the $\delta^{13}\text{C}$ values of arriving gobies but no interaction between year and species ($F_{7,237} = 1.9$, $p = 0.07$). Overall, *A. guamensis* were slightly more enriched in ^{13}C than either *L. concolor* or *S. stimpsoni* ($p < 0.05$). Accounting for species, 1998, 1999, and 2002 formed a single homogeneous subset ($p < 0.05$) whereas 1996 and 1997 formed a more enriched homogenous subset.

For $\delta^{15}\text{N}$ values, we determined a significant effect of year ($F_{4,237} = 10.15$, $p < 0.001$) and species ($F_{2,237} = 8.72$, $p < 0.001$) with a significant interaction between these two factors ($F_{7,237} = 5.60$, $p < 0.001$). The year 2002 formed one homogeneous subset with the lowest $\delta^{15}\text{N}$ values; 1996, 1997, and 1998 formed another subset with intermediate $\delta^{15}\text{N}$ values and 1999, the most enriched $\delta^{15}\text{N}$ values (Table 3). All three species formed homogeneous subsets with *S. stimpsoni* being the most depleted, *L. concolor* intermediate, and *A. guamensis* the most enriched in mean $\delta^{15}\text{N}$ values.

POM for offshore and inshore environments did not vary nor did values for epiphytic freshwater algae. Accordingly, we decided to combine values for all samples for the years for which we had the most complete data sets and which also showed the least inherent variation: 1999–2002. Given that the stable isotope values of new recruits did not closely coincide with any specific nutrient source, we felt that the most parsimonious approach was to consider an isotopic mixing model to evaluate the roles of all possible nutrient sources in an unbiased manner. Once again, we employed data only from those years for which we had data for all three dietary endpoints, 1999 and 2002. Mean isotopic endpoints and elemental concentration values for the concentration-dependent 3-source, two-isotope model of Phillips & Koch (2001) were derived from values shown in Table 4. We assumed trophic fractionation factors of 1‰ for carbon and 3.4‰ for nitrogen diet-tissue stable isotopes (Michener & Schell, 1994). This model allowed us to estimate the percent contributions from each of these sources to larval goby biomass for the year(s) of interest. Models were created by using both inshore and offshore POM for each species. Although some variation was apparent in the results, the models all suggested that newly arriving gobies derived 80–90% of their nutrients from a combination of freshwater algae and marine POM with leaf litter having a minor role (Table 4). The calculated contributions of freshwater algae and POM were similar. For example, our model for *A. guamensis* and offshore POM suggested this species might be acquiring nearly 50% of its nutrients from marine sources, about 40% from freshwater algae, and approximately 10% from leaf litter (Table 4, Fig. 3). Models using inshore POM tended to suggest a greater role for freshwater algae than offshore models.

Marine species

Reference species from the marine pelagic zone had $\delta^{13}\text{C}$ values between -15.8 and -16.9‰ and $\delta^{15}\text{N}$ values ranging from 7.1 to 12.5‰ (Table 5). As expected, marine benthic species were more enriched in their $\delta^{13}\text{C}$ values, ranging from -16.7 to -12.9‰ (Table 5). Juvenile flag-tails, that live in the lower reaches of the stream had $\delta^{13}\text{C}$ values close to other stream animals, while larger individuals that live farther out from the stream were closer to marine pelagic species in their $\delta^{13}\text{C}$ values (Table 1). $\delta^{15}\text{N}$ values approximately matched expected trophic levels. For example, in the marine pelagic grouping the large marlin, *Makaira nigricans*, had a higher $\delta^{15}\text{N}$ value than smaller scombrids such as the mackerel scad (Table 5). Among the marine benthic species the predatory hard-headed hawk fish had higher $\delta^{15}\text{N}$ ratios than the more herbivorous surgeon fishes.

Table 1. Summary of stable isotope values (mean $\pm$ SD) measured for adult resident fishes caught in Hakalau Stream, Hawai'i 1996–2002.

Species	Common name	Date	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Instream Residents					
<i>Lentipes concolor</i>	'O'opu alamo'o	1997	5	7.9 $\pm$ 0.6	-18.9 $\pm$ 1.4
		1998	8	7.8 $\pm$ 0.33	-17.7 $\pm$ 0.6
		1999	5	7.3 $\pm$ 0.43	-19.5 $\pm$ 1.0
		2002	4	6.4 $\pm$ 0.6	-19.3 $\pm$ 0.7
<i>Sicyopterus stimpsoni</i>	'O'opu nōpili	1997	6	5.5 $\pm$ 0.24	-16.5 $\pm$ 0.42
		1998	8	5.3 $\pm$ 0.27	-17.1 $\pm$ 1.02
		1999	1	5.5	-14.6
<i>Awaous guamensis</i>	'O'opu nākea	1998	3	6.9	-17.5 $\pm$ 1.3
		1999	3	7.0 $\pm$ 0.3	-18.3 $\pm$ 1.0
		2002	5	5.7 $\pm$ 0.4	-18.6 $\pm$ 0.9
<i>Eleotris sandwicensis</i>	'O'opu akupa	1998	5	7.0 $\pm$ 0.08	-18.4 $\pm$ 0.68
		1999	1	7.5	-19.6

Discussion

Initially, we had expected that freshwater fishes resident to Hakalau Stream would possess much more depleted $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than marine organisms (Chisholm *et al.*, 1982), and so provide a convenient means of evaluating the relative contribution of marine-derived nutrients to the diet of larval amphidromous fishes. Instead, we found a significant algal contribution to the freshwater food web of this model stream which resulted in stream resident fishes more enriched in ^{13}C than expected from a C-3 temperate continental freshwater food web. This effect reduced from expected the carbon isotopic difference between those nutrients available to arriving larval gobies at the stream compared with marine POM. Nonetheless, we conservatively examined those arriving gobies for the years for which we had contemporary data on all of the three assumed sources of primary production available from the stream and from the marine environment. That analysis provided evidence that recruiting post-larval amphidromous gobiid fishes receive significant quantities of nutrients from the freshwater stream prior to entering the stream *per se*. Their stable isotope ratios were remarkably similar to that of stream resident adults which in turn were notably close to those of the large quantities of freshwater algae found in these streams. If true, then nutrient plumes exuding from freshwater streams in oceanic islands such as Hawai'i likely represent an important feeding and staging area for the many amphidromous fishes that populate these systems. This finding raises the intriguing possibility that Hawaiian freshwater ecosystems serve as a critical source of nutrients for larval amphidromous fishes while at sea, and that these systems have a high reliance on algae production.

Our use of a dual-isotope approach and the large number and variety of samples we collected over a five year period gives us confidence that our isotopic values and interpretations are not influenced by any short-term fluctuations in stream or inshore food web isotopic signatures. We are also confident that recruits we sampled had just arrived from the ocean and reflected larval values for several reasons. All species of new recruits had similar isotope ratios whether collected right at the front or 100 m upstream and were found to contain some diatoms of estuarine origin. Notably, the stable isotope ratios of these recruiting fishes did not appear to change during their initial short movement inland. Our data also suggest that this movement is very rapid, although perhaps not quite as rapid as suggested by Tate (1997).

Table 2. Stable isotope values (mean±SD) and elemental concentrations of dietary endpoints used in the two-isotope, three-input mixing model used in this study.

Common name	[C]	[N]	Site	Year	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
1. Marine Production							
POM	16%	1.7%	inshore	2002	3	5.5±0.3	-20.0±0.2
POM	16%	1.7%	offshore	2002	3	3.5±0.9	-19.5±0.7
POM*	16%	1.7%	inshore	1999	3	5.0±0.2	-19.3±0.2
2. Stream Production							
Leaf Litter	8%	1.7%	Hakalau	1999	3	1.8±0.8	-26.7±0.4
	8%	1.7%	Hakalau	2002	6	-2.0±1.3	-28.7±1.8
Epiphytic algae	57.3%	4.5%	Hakalau	1999	3	2.5±0.7	-16.6±0.3
			Hakalau	2002	1	2.4	-16.8

* derived from microzooplankton sample assuming 3.4‰ and 1‰ isotopic fractionation from diet for nitrogen and carbon, respectively.

Table 3. Summary of stable isotope values (mean±SD) measured for arriving migrant gobies at Hakalau Stream, 1996-2002. New arrivals represent fish collected at the interface and at the bridge site.

Species	Common name	Date	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
<i>Lentipes concolor</i>	'o'opu alamo'o	1996	8	6.9±0.41	-17.9±0.25
		1997	5	6.5±0.22	-18.0±0.1
		1998	17	6.4±0.98	-18.2±0.93
		1999	28	6.9±0.5	-18.9±0.8
		2002	4	6.4±0.5	-18.6±0.3
<i>Sicyopterus stimpsoni</i>	'o'opu nōpili	1996	23	6.1±0.4	-18.3±0.27
		1997	7	6.5±0.48	-18.3±0.07
		1998	27	5.8±0.52	-18.5±0.56
		1999	17	6.8±0.8	-19.0±0.7
		2002	2	6.2±0.5	-18.8±0.3
<i>Awaous guamensis</i>	'o'opu nākea	1996	19	6.5±0.28	17.6±0.46
		1997	15	6.5±0.24	-17.3±0.5
		1998	17	6.7±0.23	-17.3±0.3
		1999	40	7.1±0.6	-18.9±0.5
		2002	9	6.0±0.5	-18.5±0.4
<i>Eleotris sandwicensis</i>	'o'opu 'akupa	1999	1	7.1	-23.5

Unfortunately, little comparative oceanic stable isotope data exists for primary production and zooplankton about the Hawaiian Archipelago (E.A. Laws, B. Popp, pers. comm.). In particular, we recognize that a rigorous test of our hypothesis is not possible without comparative isotopic data from purely marine fish larvae of similar ecology. Laws *et al.* (1999) reported $\delta^{15}\text{N}$ values for filtered particulate matter at the most marine sites off Honolulu to be 3.5‰, similar to our mean value of 3.0‰. However, stable-nitrogen isotope values of invertebrates and POM of the order of 8‰ have also been reported previously and are expected in nitrate-poor tropical waters (Wada & Hattori, 1976; Gould *et al.*, 1997). The carbon isotopic values we measured for marine POM were in gener-

Table 4. Results of mixing model estimates of percent biomass contribution to diets of returning larval gobies at Hakalau Stream, Hawai‘i.

Species	n	Year	% marine	% stream leaf litter	% stream algae
Inshore Model					
<i>A. guamensis</i>	40	1999	50.3	16.3	33.4
<i>S. stimpsoni</i>	17		38.9	20.2	40.9
<i>L. concolor</i>	28		42.7	18.4	38.8
<i>A. guamensis</i>	9	2002	20.8	16.8	62.4
<i>S. stimpsoni</i>	2		27.2	16.7	56.1
<i>L. concolor</i>	4		31.9	14.3	53.8
Offshore Model					
<i>A. guamensis</i>	9	2002	46.3	11.8	41.9
<i>S. stimpsoni</i>	2		60.1	9.9	30.0
<i>L. concolor</i>	4		70.7	6.6	22.7

al agreement with those found previously for similar latitudes of the Pacific Ocean (Rau *et al.*, 1982, 1989; France, 1995). Parnell (2001) measured larval bivalves (*Spondylus tenebrosus*) off Honolulu and found they averaged about 3.6 ‰ for $\delta^{15}\text{N}$ and -20.2 ‰ for $\delta^{13}\text{C}$ values. These larvae were thus substantially depleted in both isotopes compared to our “arriving” larvae. Finally, the stable isotope values we measured from several species of marine benthic and pelagic fishes (data not shown) bore no resemblance to those of recruiting gobies, although such fishes are not likely to be ecological equivalents to the larval gobioids we examined. Together, the most parsimonious interpretation of our results is that larval amphidromous gobioid fishes derive nutrients from some of the same terrestrial sources as the stream resident fishes. If true, the mouth of Hakalau Stream, and likely that of many other tropic island streams, function as important nursery regions for the amphidromous fish that spend most of their lives within them.

Examining the stable isotope ratios of stream residents, we find clear evidence both for an important role of algae in Hawaiian freshwater ecosystems and dietary specialization by adults. The stable isotope ratios of adults fell between that of detritus and algae. The size of these adults suggested they had been in freshwater many (3–5) years (Ego, 1956). Although all recruits arrived in fresh water with similar isotope values, some divergence in these values was observed with age and suggested niche specialization similar to that indicated by Kido (1997). In particular, *S. stimpsoni* became relatively enriched in ^{13}C and depleted in ^{15}N . This agrees with observations that while all three gobioid species have overlapping omnivorous food habits which include algae, various invertebrates, and detritus (Ego, 1956; Lau, 1973; Kido, 1996, 1997), *S. stimpsoni* specializes in algae (Kido, 1997). Somewhat surprising was our finding of relatively higher $\delta^{15}\text{N}$ values in *L. concolor*, which suggest that it is the most carnivorous of these species, a possibility that was also raised in a cursory study by Lau (1973). Our finding that adults of these species as a whole rely heavily upon an algal-based food web throughout the Hawaiian Islands makes good sense given the high volume of algal material found in these systems; these results lend biochemical support to the gut analysis work of Kido (1996, 1997) from a stream in the island of Kaua‘i.

This study provides biochemical evidence that amphidromous gobioid fishes aggregate at river mouths some time prior to their entry where they feed actively and acquire considerable body mass. Whether this means that many larval fish spend their entire marine existence within freshwater nutrient plumes, or rather they stage at river mouths after a period of drifting offshore is as yet unclear. A study of $\delta^{15}\text{N}$ changes in a landlocked Japanese goby suggests that half-life elemental turnover of this isotope in migrants is likely on the order of a month and associated with growth (Maruyama *et al.*, 2001); it seems likely that Hawaiian gobies whose stable isotope values appear to be at least 50‰ fresh water in origin, may spend at least a month in front of river mouths before entering. The similarity of the stable isotope values of all three species of recruits suggests that they likely consume

Table 5. Stable carbon and nitrogen isotope values of muscle tissue from various marine and marine-associated species from Hawai'i.

Species	Common name	n	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{15}\text{N} \pm \text{SD}$
Marine Pelagic:				
<i>Etilis carbunculus</i>	Ruby-colored snapper	1	-16.9	12.0
<i>Thunnus albacares</i>	Yellowfin tuna	3	-16.8	10.0
<i>Katsuwonus pelamis</i>	Skipjack tuna	1	-16.6	9.8
<i>Aphareus rutilans</i>	Ironjaw snapper	1	-16.4	9.5
<i>Coryphaena hippurus</i>	Mahimahi	2	-16.6	9.0
<i>Acanthocybium solandri</i>	Wahoo	1	-16.8	8.7
<i>Trachiurops crumenophthalmus</i>	Big-eyed scad	10	16.7±0.12	8.4±0.2
<i>Decapterus pinnulatus</i>	Mackerel scad	1	-16.8	7.5
<i>Stenoteuthis oualaniensis</i>	Deepwater squid	1	-16.4	7.1
<i>Makaira nigricans</i>	Blue marlin	1	-15.8	12.5
Marine Benthic:				
<i>Cirrhitus pinnulatus</i>	Hard-headed hawk fish	5	-12.9±0.6	10.5±0.2
<i>Acanthurus sandvicensis</i>	Sandwich Island surgeon	5	-13.3±1.7	8.6±0.4
<i>Acanthurus achilles</i>	Achilles surgeon fish	5	-14.6±0.4	7.8±0.6
<i>Acanthurus dussumieri</i>	Dussumier's surgeon fish	5	-16.7±2.4	8.6±0.8
<i>Parupeneus multifasciatus</i>	Red & black banded goat fish	5	-16.7±0.2	8.7±0.3
<i>Polydactylus sexfilis</i>	Pacific thread-fin	1	-16.4	9.2
Stream Mouth:				
<i>Kuhlia xenura</i>	Hawaiian flag-tail (30 mm)	9	-17.5±0.1	4.7±0.3
<i>Polydactylus sexfilis</i>	Pacific thread-fin	1	-18.3	6.4

very similar food in similar locations, with *A. guamensis* (whose $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were both slightly higher) perhaps being located slightly more inshore than the other species. In any case, that these larval fish do not distribute themselves widely as commonly imagined prior to stream entry, makes some ecological sense given the enormity of the Pacific Ocean and the fact that it is nutrient poor, while freshwater streams are nutrient-rich.

Our suggestion that larval gobies spend some time in river mouths, does not conflict with findings of previous studies. Thus, while Radtke *et al.* (1988, 1996) employed stable-isotopic analyses of the inorganic fraction of Hawaiian goby otoliths to describe an early, several-month long, larval phase in salt water, their data do not preclude that this marine stage might concentrate in diluted river nutrient plumes. This emphasises key differences in interpretations based on isotopic analyses of otoliths which give information on the nature of the elemental environment in which the fish spent time and the isotopic analyses of metabolically active fish tissues which give information on the nutritional sources contributing to fish biomass. Notably, Radtke *et al.* (1996) note surprisingly little variance in the age of 'larval settlement', an observation suggesting to us that many new recruits may not be coming from great distances. Similarly, while both Fitzsimons *et al.* (1990) and Zink *et al.* (1996) note a lack of genetic evidence for specific populations of gobies between islands, their results could be explained by as few as one recruit per generation drifting between streams (Zink, pers. comm.).

Conclusions

We have demonstrated evidence to suspect that the larvae of amphidromous Hawaiian fish, and likely those from other tropical island archipelagos, spend the majority of their early lives in river plumes and subsist on material associated with freshwater food webs. Further, we suggest that these food webs may derive much of their energy from algal primary production. Lastly, it seems reason-

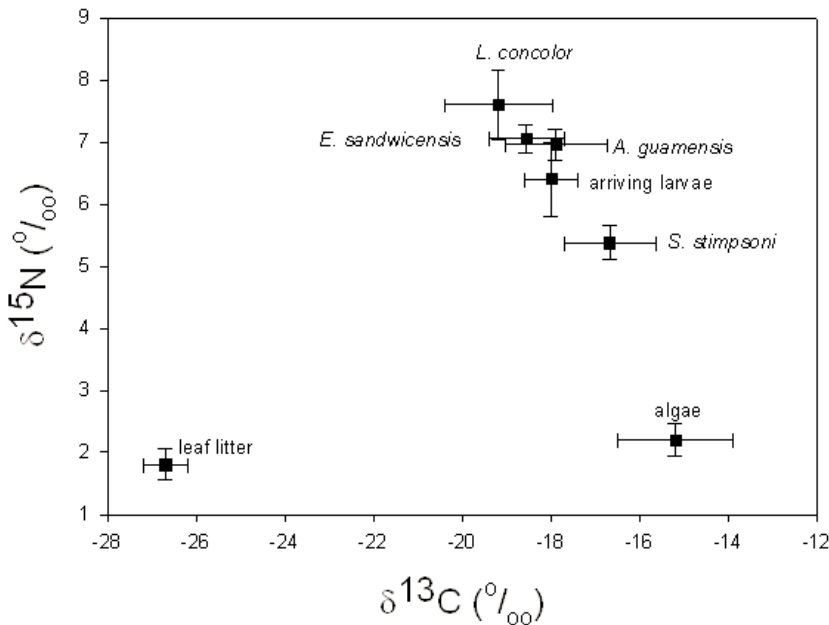


Figure 2. Relative isotopic positions of stream resident adult gobies and returning migrant larval gobies (all species combined) relative to Hakalau Stream primary production sources (1999–2002). This figure suggests a stronger algal (autochthonous) than leaf litter (allochthonous) input to the stream food web. For simplicity, only leaf litter values for 1999 shown. See Tables 1 and 2 for individual isotope values.

able to hypothesize that the many species of amphidromous invertebrates likely pursue similar life history strategies which rely upon freshwater stream productivity while at sea, yet still allowing for some dispersal. If true, these hypotheses have important implications for both the ecology and management of these unique tropical ecosystems, many of which are now threatened by development and water removal, and previously were suspected to derive their energy from detritus and to have no role as nursery habitat. Instead, our data now suggest that each freshwater stream and its associated nutrient plume within these systems should be considered a coherent ecological unit that supports indigenous amphidromous organisms both as larvae and adults. Direct tests of these ideas by evaluating the food webs of these systems with a combination of laboratory feeding and rearing experiments together with the measurement of larvae of purely marine species off Hawai'i are now warranted.

The inherent problems involved with resolving marine and algal-based freshwater food webs due to overlap in stable isotope signatures suggests that future isotope studies should make use of other stable isotope measurements in addition to those of elements used here. In particular, we recommend the use of deuterium (δD) and sulphur ($\delta^{34}\text{S}$) isotope measurements in order to obtain tracers that may be independent of the nature of the freshwater algal vs. marine POM constraint.

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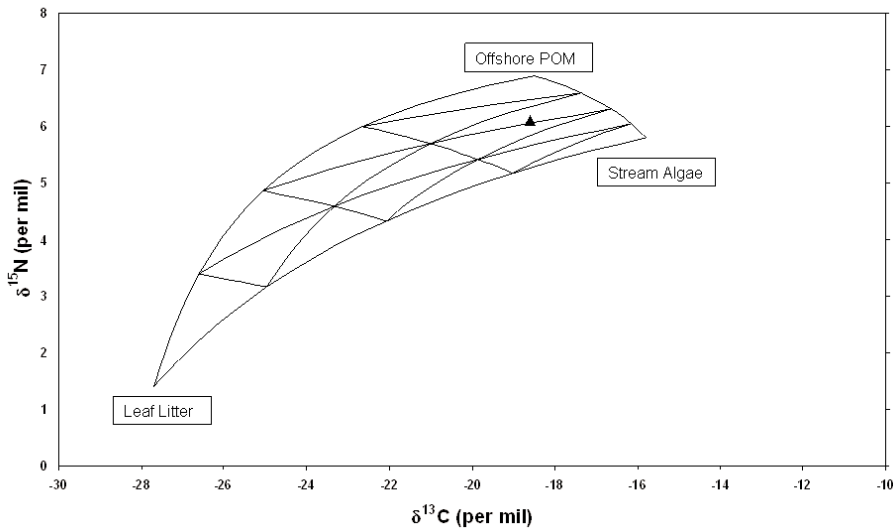


Figure 3. Example of the three-source, two-isotope mixing model used to estimate mean contribution of marine POM, instream algae and leaf litter to diets of returning larval gobies at Hakalau Stream. This figure shows the model parameters for 2002 using the offshore POM endpoint and larval returning *A. guamensis*. Note that the endpoint values represent the isotopic value of a hypothetical larva consuming 100% of this diet.

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