Habitat coupling writ large: pelagic-derived materials fuel benthivorous macroalgal reef fishes in an upwelling zone

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Abstract. Coastal marine upwelling famously supports elevated levels of pelagic biological production, but can also subsidize production in inshore habitats via pelagic-benthic coupling. Consumers inhabiting macroalgae-dominated rocky reef habitats are often considered to be members of a food web fuelled by energy derived from benthic primary production; conversely, they may also be subsidized by materials transported from pelagic habitats. Here, we used stable isotopes ($\delta^{13}$C, $\delta^{15}$N) to examine the relative contribution of pelagic and benthic materials to an ecologically and economically important benthivorous fish assemblage inhabiting subtidal macroalgae-dominated reefs along ~1,000 km of the northern Chilean coast where coastal upwelling is active. Fish were isotopically most similar to the pelagic pathway and Bayesian mixing models indicated that production of benthivorous fish was dominated (median 98%, range 69–99%) by pelagic-derived C and N. Although the mechanism by which these materials enter the benthic food web remains unknown, our results clearly highlight the importance of pelagic-benthic coupling in the region. The scale of this subsidy has substantial implications for our basic understanding of ecosystem functioning and the management of nearshore habitats in northern Chile and other upwelling zones worldwide.

Key words: cryptic; ecosystem functioning; kelp forest; mixing models; pelagic-benthic coupling; stable isotope analysis; trophic subsidies.

INTRODUCTION

Eastern-boundary currents are characterized by the upwelling of cold, nutrient-rich sub-surface waters that support heightened biological productivity in pelagic marine habitats (Chavez and Messi 2009). The Peru-Chile upwelling system supports the World’s most productive industrial fishery, targeting pelagic fishes (Thiel et al. 2007, Chavez and Messi 2009). However, human demands for fish in the region are largely sustained by small-scale inshore fisheries (Thiel et al. 2007), targeting benthivorous fishes (Pérez-Matus et al. 2012; Data S1) inhabiting subtidal macroalgae-dominated reefs, where they can play key functional roles (Ortiz 2008).

Consumers inhabiting macroalgae-dominated reefs are often assumed to belong to a foodweb largely fed by autochthonous organic material derived from benthic primary production (Fariña et al. 2008, Ortiz 2008) i.e., macroalgae and to a lesser extent periphytic microalgae (but see Miller and Page 2012). However, given the scale of pelagic primary production in upwelling zones such as northern Chile (Thiel et al. 2007), secondary production in macroalgae-dominated reef habitats is also likely subsidized by pelagic-derived allochthonous materials (e.g., phytoplankton and associated detritus). Pelagic-derived materials transported into benthic habitats are potentially consumed and assimilated by benthic primary consumers and then transferred to higher trophic levels in the benthic
food web, through pelagic-benthic coupling (Grebmeier et al. 1988). Inshore habitats in Chile can show increased productivity due to upwelling (Boisman et al. 1987), as seen in other regions, but the scale of any trophic subsidy remains unknown. Quantifying such connectivity between open ocean and coastal habitats is difficult, but is required for our fundamental understanding and management of coastal ecosystems (Polis et al. 1997).

We examined whether a benthivorous fish assemblage inhabiting macroalgal-dominated reef habitats were subsidized by pelagic-derived materials, using stable isotope analysis (Peterson 1999). In marine systems, pelagic and benthic sources of carbon typically (but not always) have distinct δ13C values, with pelagic-derived carbon being 13C-depleted relative to carbon originating from benthic primary producers (France 1995, Peterson 1999). Although nitrogen isotopes are typically used to estimate consumer trophic position (Post 2002), they also provide a means to detect the presence of 15N-enriched nitrogen derived from upwelling (Liu and Kaplan 1989). Stable isotopes can be used as a tracer of material flux across pelagic and benthic habitats, and by using mixing models (Parnell et al. 2010), the proportional contribution of isotopically different sources of energy and nutrients to consumers can be estimated (Peterson 1999). Using these methods, we test the general hypothesis that, contrary to the established paradigm, in regions of intense and permanent upwelling conditions, pelagic-derived materials provide a significant subsidy to the macroalgae-dominated rocky reef fish assemblage.

**MATERIALS AND METHODS**

During the Austral summer (moderate El Niño conditions) and winter (moderate La Niña conditions) 2010, we sampled fishes and intertidal/subtidal invertebrates from macroalgal-dominated rocky reef habitats (depth < 20 m) from five localities situated along a latitudinal gradient of ~1,000 km in northern Chile (Fig. 1A). Primary consumers functionally associated with the consumption of either phytoplankton (filter-feeding bivalve: *Perumytilus purpuratus*, mean n per location/season = 8) or benthic macro- and macroalgae (grazing gastropods: *Echinolitorina peruviana*, *Prisogaster niger*, *Tegula atria*; mean n = 9), were collected to estimate isotopic endpoints for pelagic (bivalves) and benthic (gastropods) sources of primary production (Post 2002). We purchased ~20 adult individuals of the macroalgal reef fish assemblage including *Anisotremus scapularis*, *Cheilodactylus variegatus*, *Graus nigra*, *Hemilatjanus macrophthalma*, *Paralabrax hameralis*, *Pinguipes chilensis* and *Sicyases sanguineus* from commercial spear divers at each location. All species are benthivorous (Data S1), consuming a range of taxa associated with macroalgae/rocky reef habitats.

Muscle samples were dried (60°C, 48 h), homogenized and weighed (~0.5 mg) into tin capsules for determination of δ13C, δ15N and elemental %C : %N at the 14Chrono Centre, Queen’s University of Belfast, UK. Repeated analysis of an internal standard (IA-R041 -Alanine) showed analytical errors to be ~0.1‰ for δ13C and δ15N.

Fish δ13C data were corrected for lipid variation (Kiljunen et al. 2006) due to spatial differences in C : N values (GLM: F4, 165 = 2.9, P = 0.02). Invertebrate C : N values showed no apparent variation either by location (F4, 151 = 2.4, P = 0.05) or functional group (F1, 151 = 0.48, P = 0.49), and were low (mean ± SD = 3.2 ± 0.6, n = 157), and as such were not corrected for lipid variation.

We examined variation in baseline and fish δ15N–δ13C values using multivariate analysis of variance (PERMANOVA), based on a Euclidean similarity matrix of untransformed δ15N–δ13C data (Anderson et al. 2008). In order to examine whether baseline variation existed in carbon and nitrogen stable isotope values between pelagic and benthic pathways, location and season, we examined the relative influence of these factors (Location, Season and Functional Group on invertebrate δ15N and δ13C values using a 3-way permutational (nP = 9,999) multivariate analysis of variance (PERMANOVA), based on a Euclidean similarity matrix of untransformed δ15N–δ13C data. The 3-way interaction term (Location×Season×Functional Group) was significant (Pseudo-F4, 143 = 5.28, P = 0.0001), indicating that baseline shifts were not consistent across locations, seasons or functional groups (see Appendix S1: Table S1). As such, we compared the relative influence of Season and invertebrate Functional Group at individual locations using 2-way PERMANOVA. As δ15N–δ13C baselines varied, comparisons of fish δ15N–δ13C values were limited to 1-way PERMANOVAs examining potential seasonal differences at different locations.

The Bayesian mixing model SIAR (Parnell et al. 2010) was used to estimate the relative contribution of pelagic- and benthic-derived materials to fish somatic tissues (muscle). Pelagic and benthic endpoints were estimated separately for each location and season using filter-feeding and grazing invertebrate primary consumers, respectively. SIAR, like other Bayesian stable isotope mixing models (SIMMS: see Parnell et al. 2013 for a description), estimates the relative contribution of different sources to consumers (mixture), and includes information on individual consumer δ13C and δ15N values, as well as mean ± SD of δ13C and δ15N for sources (in this case invertebrate primary consumers) and for trophic enrichment factors (TEFs), the isotopic difference between a consumer and the source of the C and N in its tissues. By including errors associated with fractionation and sources, and allowing these errors to propagate throughout the model, Bayesian SIMMs provide a more robust estimate of uncertainties associated with different source contributions than the simple linear mixing models used previously. Here we assume that the system is fueled by only two sources—pelagic and benthic primary production, with no contribution from other sources, e.g., terrestrial-derived materials, which
are often included in studies of coastal food webs. Given the location of the sampling locations in northern Chile, where terrestrial vegetation and riverine inputs are extremely limited (or even completely absent), we feel that this assumption is justified. Trophic enrichment factors were 2.6 for $^{13}C$ and 5.8 for $^{15}N$ (McCutchan et al. 2003), allowing for an estimated difference in two trophic levels between invertebrates (primary consumers) and fish. TEFs (and therefore the results of SIMMs) are sensitive to trophic level differences between the mixture and sources, and can vary considerably according to feeding mode and food quality. We are confident that the invertebrates we used as indicators of pelagic- and benthic-derived primary production, are primary consumers, and information from stomach content analysis (Data S1) indicate that the fish are tertiary consumers. We used relatively wide SD values for both $^{13}C$ and $^{15}N$, allowing the TEFs to encompass a range of likely fractionation values, increasing our confidence in mixing model results.

**RESULTS**

Pelagic and benthic primary consumers were consistently differentiated in their $^{13}C$ values (Fig. 1B; Data S2). Overall, filter feeding bivalves (mean $\pm$ SD = $-16.9 \pm 1.1\%_o$) were $^{13}C$ depleted by $4.2\%_o$ relative to grazing invertebrates ($-12.7 \pm 1.2\%_o$). Benthivorous fish ($-15.5 \pm 0.9\%_o$) were enriched in $^{13}C$ by $1.4\%_o$ relative to the pelagic endpoint represented by the filter-feeding bivalves, but were $^{13}C$ depleted by $-2.8\%_o$ to the benthic endpoint (grazing invertebrates). All consumers were relatively $^{15}N$ enriched, likely reflecting the influence of isotopically heavy, upwelling-derived N (Fig. 1B) to both pelagic and benthic primary producers. At the level of functional group, filter feeding ($15.3 \pm 1.4\%_o$) and grazing ($15.9 \pm 1.4\%_o$) invertebrate $^{15}N$ values were generally similar, but fish ($21.3 \pm 1.3\%_o$) were $^{15}N$ enriched by between $5.4\%_o$ and $6\%_o$ relative to the two groups of invertebrates. At a broad geographical scale, $^{13}C$ and $^{15}N$ values appeared qualitatively similar in summer and winter (Fig. 1B) for all functional groups, but detailed statistical comparisons revealed that baselines were not consistent and varied by Location and Season.

We compared the relative importance of Location, Season and Functional group on variation in invertebrate $^{13}C$ and $^{15}N$ values using PERMANOVA. The 3-way interaction was significant (Appendix S1: Table S1; Pseudo-$F_{3, 143} = 5.28$, $P = 0.0001$), limiting our abilities to make overall comparisons of the relative importance of the main effects (Location, Season and Functional Group). We then conducted subsequent within-location 2-way PERMANOVAs examining the influence of Season and Functional group on $^{15}N$ and $^{13}C$ variation (Appendix S1: Table S2). In two of five comparisons, there was a significant Season x Functional group interaction (Tocopilla: $F_{1, 20} = 3.78$, $P = 0.04$; Iquique: $F_{1, 40} = 15.2$, $P = 0.0002$), suggesting
that at these sites, seasonal shifts in baseline $\delta^{15}\text{N} - \delta^{13}\text{C}$ values differed between pelagic and benthic baselines. In those sites where the interaction term was not significant and could be dropped from the model, subsequent analyses showed that Functional Group had the largest effect on $\delta^{15}\text{N} - \delta^{13}\text{C}$ variation (Appendix S1: Table S2) relative to that of Season.

As baseline $\delta^{15}\text{N} - \delta^{13}\text{C}$ values showed significant differences by location and season, we restricted comparisons of fish isotope values to individual locations, i.e., we used one-way PERMANOVA to compare summer and winter fish $\delta^{15}\text{N} - \delta^{13}\text{C}$ values (Winter data were missing from Antofagasta, so this location was dropped from the analysis). There was a significant effect of season at three of the four locations ($P < 0.05$; Appendix S1: Table S3), with only fish from Coquimbo showing no evidence for seasonal shifts ($F_{1,46} = 3.29$, $P = 0.05$).

Visual examination clearly shows that the $\delta^{15}\text{N} - \delta^{13}\text{C}$ space inhabited by the benthivorous fish assemblages was closely associated with that related to the assimilation of material of a pelagic origin (see location of centroids relative to location of those of pelagic and benthic baselines in Fig. 1B). This was supported by the results of the Bayesian mixing models that showed the overwhelming contribution of pelagic-derived C and N to the assimilated diet of benthivorous fishes for each combination of location and season (Table 1: median modal pelagic contribution $= 98\%$, modal range $= 69$–99%). The modal estimated contribution of benthic-derived C and N reached more than 30% in only a single case (winter 2010 in Coquimbo).

**DISCUSSION**

We aimed to examine the relative contribution of pelagic- and benthic-derived materials to the benthivorous fish assemblage associated with macroalgal-dominated reef habitats adjacent to an active upwelling zone. Although the literature clearly shows these fishes consume benthic prey associated with macroalgal-reef habitats (see Data S1), our results show that their somatic tissues were isotopically largely derived from materials with a pelagic origin. The existence of such pelagic subsidies to benthic habitats has been long recognized (Grellmeier et al. 1988), but not to such an overwhelming scale. Our data suggest that although macroalgae provide important habitat and structure, in N Chile at least, they are not a major source of energy and nutrients to coastal benthivorous fishes and the invertebrates they consume (Miller and Page 2012), contrary to recent data (von Biela et al. 2016) suggesting that macroalgae can play an important role (15–99% contribution) in fueling kelp-associated fishes. Our data show that the basic assumption that food webs in such habitats are fueled by benthic primary production (Fariña et al. 2008, Ortiz et al. 2015) is now open to question, at least in regions where primary production is elevated, e.g., adjacent to upwelling zones.

The flux of materials across perceived ecosystem barriers is common (Polis et al. 1997), including between pelagic and neritic marine habitats in Chile (Thiel et al. 2007) and elsewhere, but the scale of the putative subsidy we have revealed was unexpected. The fishes studied here feed on benthic taxa, including decapods, amphipods, polychaetes, molluscs and fish (Data S1) that inhabit macroalgal-dominated habitats. Presumably, the pelagic-derived materials (e.g., detritus or settling plankton) enter the benthic food web through their consumption and assimilation by lower-trophic position suspension and deposit feeding benthic invertebrates, which are then consumed by higher-trophic position consumers, such as the benthivorous fishes examined here.

Our results indicate that for a large section of the northern Chilean coastline, the supply of pelagic-derived materials is such that it overwhelms the contribution of autochthonous materials originating from benthic production, even though this can be considerable (Thiel et al. 2007). We did not estimate the relative availability of benthic and pelagic materials, but estimates from the literature indicate that primary production in the pelagic and benthic habitats is in the same order of magnitude (Thiel et al. 2007).

Our conclusions rely on the assumption that our isotopic endpoints are isotopically representative of pelagic and benthic sources of primary production. We used

<table>
<thead>
<tr>
<th>Location</th>
<th>Summer 2010 Pelagic</th>
<th>Winter 2010 Pelagic</th>
<th>Summer 2010 Benthic</th>
<th>Winter 2010 Benthic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coquimbo</td>
<td>0.98 (0.88–1.0)</td>
<td>0.69 (0.55–0.96)</td>
<td>0.31 (0.03–0.45)</td>
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</tr>
<tr>
<td>Taltal</td>
<td>0.99 (0.91–1)</td>
<td>0.99 (0.81–1)</td>
<td>0.01 (0.0–0.2)</td>
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</tr>
<tr>
<td>Antofagasta</td>
<td>0.94 (0.45–1)</td>
<td>0.99 (0.91–1)</td>
<td>0.01 (0.0–0.1)</td>
<td></td>
</tr>
<tr>
<td>Tocopilla</td>
<td>0.98 (0.71–1)</td>
<td>0.94 (0.82–1)</td>
<td>0.06 (0.0–0.18)</td>
<td></td>
</tr>
<tr>
<td>Iquique</td>
<td>0.93 (0.87–0.99)</td>
<td>0.07 (0.0–0.13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Modal contributions (95% credibility limits) were estimated using the SIAR mixing model.*
relatively long-lived primary consumers (Post 2002, Mallela and Harrod 2008) to provide a representation of the pelagic and benthic primary production pathways (France 1995). Our study, like others (Mallela and Harrod 2008) showed filter feeding bivalves to be consistently 13C depleted relative to grazing gastropods. Although we did not directly measure isotopes from primary producers in this study, a study undertaken in the Antofagasta region by our group (Reddin et al. 2015) and subsequent unpublished work on the same indicator taxa supports our assumption. Bivalves sampled in the identical location as those shown here were isotopically more similar to POM, whilst grazing gastropods had δ13C and δ15N values close to that of macroalgae. There is clearly the possibility that both some kelp-derived material is consumed by filter feeding bivalves and that grazing gastropods can consume pelagic-derived detritus, but given the consistent isotopic differences between the two functional groups we feel confident that, as in freshwater systems (Post 2002), these taxa provide a reliable indicator of pelagic and benthic pathways in coastal systems.

Trophic subsidies play important roles in ecosystem function (Polis et al. 1997), and coastal pelagic upwelling can influence the fundamental ecology of neritic and intertidal ecosystems, including biological productivity (Bosman et al. 1987), interspecific interactions (Nielsen and Navarrete 2004), fish growth (Pulgar et al. 2013), consumer diet and community structure (Reddin et al. 2015), and here, our data indicate that the bottom up supply of basal resources drives secondary production in macroalgae-dominated reef foodwebs. Our data highlight the complexity of open ecosystems (Polis et al. 1997), and unveil a previously unrecognized large-scale trophic subsidy in the eastern South Pacific. There is a pressing need to characterize the mechanism by which allochthonous materials enter the neritic foodweb and to examine whether such subsidies exist in other major upwelling zones worldwide and how this affects our management of coastal resources.

Acknowledgments

CONICYT (Chile) for funding (PAI Grants MEC 80095003; MEL 81105006; FONDECYT 1151515). J. Villanueva for help in the field. M. Thiel & M.E. Oliva for project support. UASIF is funded by FONDEQUIP-V-EQM160171.

Literature Cited


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