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HOW SPECIALIZED ARE THE DIETS OF NORTHEASTERN PACIFIC SPONGE-EATING DORID NUDIBRANCHS?

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ABSTRACT

Cryptobranch dorids are typically thought to have specialized diets limited by prey skeletal architecture and chemistry, but recorded diets for some species are broader than expected. Few studies have directly compared prey use with prey availability over multiple sites to test the dietary range of separate populations. Diets of dorids at sites in British Columbia, Canada were not simply related to the richness and diversity of the local sponge assemblage, partly because all dorids avoided the most common sponges and instead consumed rare, inconspicuous species. At each site, three dorid species (*Cadlina luteomarginata*, *Diaulula sandiegensis* and *Peltodoris nobilis*) consumed multiple species, while two others (*Doris montereyensis* and *D. odhneri*) each consumed one species almost exclusively, so total dietary ranges may sometimes reflect true oligophages and sometimes mosaics of stenophage populations. Diets in all cases shifted greatly among sites and geographic regions and included sponges with different skeletal types, indicating that all species are nonstereotyped specialists. The diets of these dorids may be determined primarily by haphazard encounters among scarce or patchy palatable prey, with other prey attributes playing a lesser role.

INTRODUCTION

Cryptobranch dorid nudibranchs are the most diverse group of opisthobranchs, with *c.* 2000 species (Valdés, 2004). They are a key group for studying the evolution of chemical defence and feeding specialization in the marine environment (Faulkner & Ghiselin, 1983; Karuso, 1987; Cimino & Ghiselin, 1999; Wägele, Ballesteros & Avila, 2006; Rudman & Bergquist, 2007). Most cryptobranch dorids are described as feeding specialists, and several hypotheses have been proposed to explain the evolution of their diets, including the need for sequestered chemical defence (Faulkner & Ghiselin, 1983; Cimino & Ghiselin, 1999) and the ability to handle skeletal architecture of their prey (Bloom, 1976, 1981; Cattaneo-Vietti & Balduzzi, 1991).

However, for several reasons, this view of cryptobranch dorids as feeding specialists appears to be based partially on inadequate data (Todd, Lambert & Davies, 2001). First, most previous studies of dorid diets have only recorded prey consumed, although determining prey preference also requires knowledge of the prey abundance in the environment, because diet breadth is well known to increase as preferred prey become scarce (Hughes, 1980; Wulff, 2006). Such preference studies have only been done on two species. One (*Peltodoris atromaculata*) consumes only two sponges (Gemballa & Schermutzki, 2004). Another (*Platydoris argo*), a 'non-stereotyped specialist', consumes a wide range of prey and changes its diet depending on prey availability (Megina *et al.*, 2002). Secondly, seemingly polyphagous species can actually be comprised of more specialized populations (Todd *et al.*, 2001; Sotka, 2005; Thompson, 2005), but most studies have combined data from multiple field sites. Thirdly, Bloom's (1976, 1981) guild hypothesis—that cryptobranch dorids with a caecum consume sponges with disorganized skeletons, while acecate dorids consume sponges with organized skeletons, and both have radular teeth matched to their prey skeletons—has been contradicted by new evidence. All cryptobranch dorids, except the radula-less dorids, possess a caecum (Valdés, 2002); radular teeth are phenotypically plastic even within the same row (Cattaneo-Vietti & Balduzzi, 1991); and there are prey records of dorid species from each guild consuming sponges of the 'wrong' skeletal type (McDonald & Nybakken, 1997).

Diets of the common dorids of the Northeastern Pacific that eat spiculated demosponges are arguably the best investigated across a wide geographic range (Bloom, 1981; Hellou, Andersen & Thompson, 1982; Thompson *et al.*, 1982). However, in none of these studies were sponge abundances recorded and there are few published data on shallow-subtidal assemblages of demosponges in this region. Therefore, I documented abundance and diversity of sponges, and their usage as prey by dorids, from several field sites in British Columbia, Canada to determine (1) how prey availability affects diet and (2) if prey range matches Bloom's guild hypothesis.

MATERIAL AND METHODS

Study sites

I surveyed three sites in the western part of Trevor Channel (Barkley Sound, British Columbia, Canada), representing a range of communities, in the autumn of 1999 (Fig. 1). Dixon Reef (48° 51′ 24″ N, 125° 07′ 06″ W; abbreviated DXR) is a bedrock/boulder reef approx. 100 m in diameter with a medium wave exposure (Arsenault, Marchinko & Palmer, 2001), separated from the shore by approx. 400 m of mixed mud and sand bottom. Scott's Bay (SB: 48° 50′ 06″ N, 125° 08′ 48″ W) was the least wave-exposed site, with a substrate of mostly bedrock to large boulders, grading from kelp beds to open rock and overhangs, then to a sandy bottom with low slope at 12 m depth. Seppings Island West (SW: 48° 50′ 30″ N, 125° 12′ 30″ W) was the most wave-exposed site, with a substrate predominantly of open bedrock with occasional boulders and cobble, continuous with several reefs and shallower areas. Although predation risk can affect prey choice (Krebs & Davies, 1997), all sites were observed to have similar densities of generalist molluscivores: sea stars (*Pycnopodia helianthoides* and occasional *Solaster* sp.), fish (greenling and rockfish) and large crabs (primarily *Cancer* spp.), and there were no known specialist predators of opisthobranchs at these sites.

Description of sponge assemblages

Sponge availability and use were recorded along 3–5 belt transects per site at approx. 10 m depth. Species and area of each sponge patch, estimated by using a ruler against the substrate, was recorded in 0.25 m^2-square quadrats (Pringle, 1984) at randomly determined points (random numbers of fin beats) along the transects. For any sponge not readily identifiable in the field, a sample was transferred to a coded mesh-bottomed vial and returned to the laboratory for identification. Sites were sampled until at least 30 quadrats were complete and, if possible, over 15 individuals each of at least two species of dorid had been collected.

Sponge samples were stored in flowing natural seawater at Bamfield Marine Station in mesh-bottomed vials. Samples were identified using standard field guides and methods (Smith & Carlton, 1975; Kozloff, 1996). Because sponge taxonomy is still in a state of flux, Table 1 gives the names from these guides and the current taxonomy used in this paper. Any specimen identifiable with these field guides was counted as a separate species, even if the species was not officially described. For specimens that could not be identified using available guides, similar specimens were lumped together as an unknown species.

Several measures of assemblage richness were used to ensure that sampling adequately reflected the available demosponge diversity. Unknowns were counted as separate species for both richness and diversity measures, to avoid underestimating these variables. For comparability with other studies, I calculated sponge diversity directly using the Shannon index and K-dominance curves (Krebs, 1998). Direct sampling of communities may underestimate species richness if rare species are likely to be missed and so nonparametric estimators may provide better estimates of the true community richness (Magurran, 2004). I chose the Jack2 estimator, which has been characterized in a range of simulation studies and performs well at relatively small sample sizes (Magurran, 2004). This was calculated with EstimateS (Colwell, 2009) using 100 randomization runs with complete replacement.

Prey use by nudibranchs

Faecal analysis is a nonlethal sampling method that can detect rare prey items likely to be missed in field observations (Bloom,

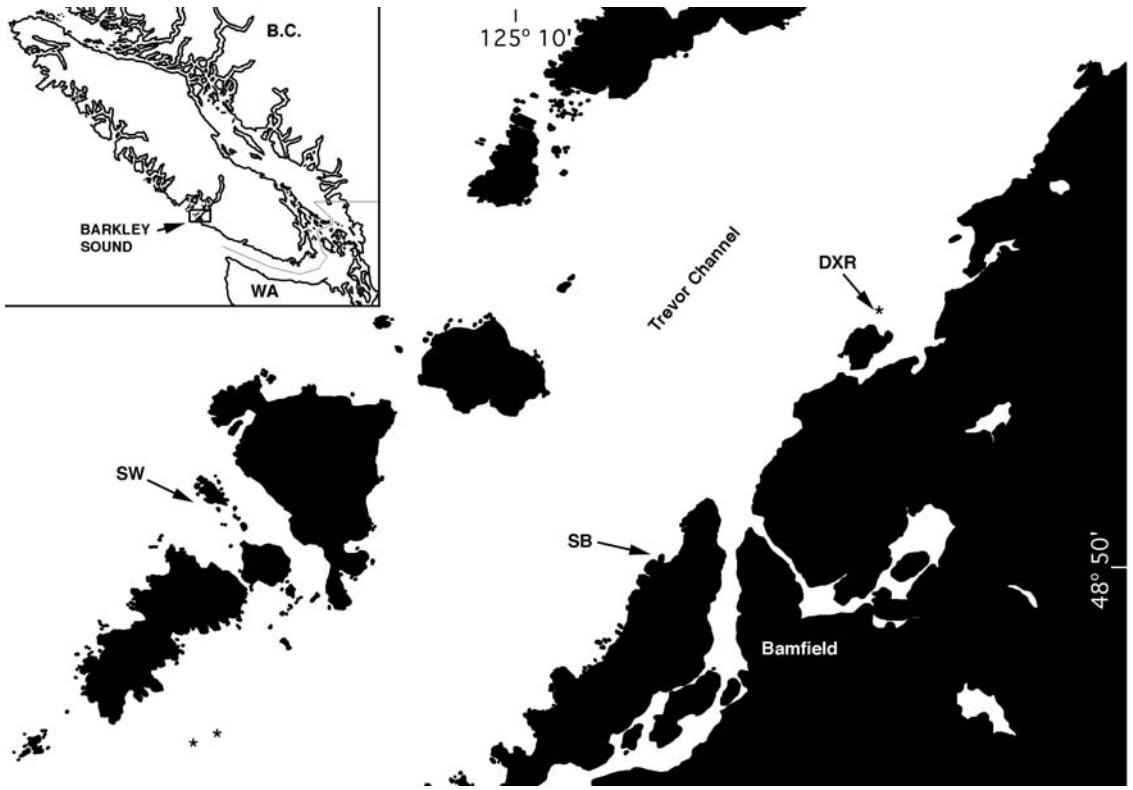

Figure 1. Map of field sites in Barkley Sound, British Columbia (B.C.) for diet study. Abbreviations: DXR, Dixon Reef; SB, Scott's Bay; SW, Seppings Island, West. North is to the top of the map. Rectangle in inset shows approximate location of larger map.

Table 1. Sponge species mentioned in the text, cross-referenced to the names under which they were identified

Sponge	Authority	Kozloff (1996) species name	CODE
Antho (Acarnia) lambeii	(Burton, 1935)	*Plocamilla lambeii*	ALAM
Clathria pennata	(Lambe, 1895)	*Ophlitaspongia pennata*	CPEN
Cliona californiana	De Laubenfels, 1932	*Cliona celata* var. *californica*	CCAL
Halichondria panicea	(Pallas, 1766)	*Halichondria panicea*	HPAN
Haliclona (Reniera) sp.	Kozloff, 1996	*Toxadocia* sp.	HRS
Haliclona cf. *cinerea*	Kozloff, 1996	*Haliclona* cf. *permollis*	HCC
Haliclona cf. *foraminosa*	Kozloff, 1996	*Adocia* cf. *Reniera foraminosa*	HCF
Haliclona gellindra	(De Laubenfels, 1932)	*Adocia gellindra*	HGEL
Hamacantha (Vomerula) hyaloderma	(De Laubenfels, 1932)	*Zygherpe hyaloderma*	HHYA
Hymedesmia sp. A	Kozloff, 1996	*Hymedesmia* sp. A	HSA
Hymeniacidon ungodon	De Laubenfels, 1932	*Hymeniacidon ungodon*	HUNG
Isodictya rigida	(Lambe, 1893)	*Neoesperiopsis rigida*	IRIG
Lissodendoryx firma	(Lambe, 1895)	*Lissodendoryx firma*	LFIR
Mycale sp.	Kozloff, 1996	*Mycale* sp.	MSP
Myxilla incrustans	(Johnston, 1842)	*Myxilla incrustans*	MINC
Neopetrosia vanilla	(De Laubenfels, 1930)	*Xestospongia vanilla*	NVAN
Pachychalina sp.	Kozloff, 1996	*Pachychalina* sp.	PSP
Plocamionida lyoni	Bakus, 1966	*Hymendectyon lyoni*	PLYO
Suberites sp.	Kozloff, 1996	*Suberites* sp.	SSP
Weberella n. sp.	Austin, 1985	*Weberella* new species	WNS

For taxonomic authority, species lacking formal taxonomic descriptions are referenced to the guide used for that diagnosis.

1981; Kitting, 1981; Gemballa & Schermutzki, 2004; Wulff, 2006). All cryptobranch dorids found within 1 m of each transect were brought to Bamfield Marine Station and kept without food in 1-l mesh-sided cages in flowing seawater for several days. Faeces were transferred to labelled vials until identification and nudibranchs were then returned to their original field sites. A representative mix of collected faeces was smeared on a clean glass slide and digested with concentrated nitric acid to obtain spicules, and sponges were identified as previously described. Samples were classified as 'empty gut' if they had too few spicules to identify accurately (<50 whole spicules). Some spicule profiles were best explained by the presence of two or more sponge species. Several *Mycale* species have identical spicule types with only slightly differing lengths (Kozloff, 1996); these could not be reliably distinguished in faecal samples, so all *Mycale* were treated as one species. However, because one nudibranch individual consumed *Mycale* in this study, this had a negligible effect on results. Samples not matching spicule profiles of sponges present at field sites, or described in the literature, were recorded as 'Unknown'. One nudibranch, *Cadlina luteomarginata*, is known to consume unspiculated demosponges (McDonald & Nybakken, 1997) and these are essentially undetectable in faeces. Although these sponges were not found at any of the field sites, diet diversity of *C. luteomarginata* could potentially be underestimated using this method.

The relative volume of prey in each dorid's diet cannot be determined from faecal spicules alone, so the number of 'isolations', or frequency of occurrence, is more appropriate (Hyslop, 1980). This measure does not describe the diet of an individual animal, but shows how uniformly the population selects a prey item without indicating importance with respect to other prey (Tirasin & Jørgensen, 1999). As long as there are no individuals with an unusually high number of prey species in their faeces, this is sufficient for matching observed diets against prey abundance. However, just as measures of percent cover can exceed 100%, the number of isolations can exceed the number of individuals sampled. Completeness of diet sampling can be tested by constructing species and diversity accumulation curves (Katona & Altbäcker, 2002; Naya, Arim & Vargas, 2002). These were calculated with EstimateS ($S_{Observed}$ Mau Tau; 50 runs with replacement; Colwell, 2009), treating individual dorids as 'quadrats' and isolations from faeces as 'incidences'. Only species with multiple prey species per site were included in this analysis.

To compare sponge abundance with use by nudibranchs, I used Pearre's C index, a 2×2 χ^2-based measure of selectivity that is simple to interpret (range -1 to 1, values above 0 mean positive selection, values below 0 mean avoidance) and applicable to small sample sizes (Pearre, 1982). This index behaves less reliably if the numbers of prey available and prey consumed are not similar in magnitude (Pearre, 1982), so data were transformed to percentages (as often done for plankton studies; Giesecke & González, 2008; Salonen, Urho & Engström-Öst, 2009). Dorid/site combinations with less than five isolations were excluded from analysis.

To compare how diet breadth might vary geographically, data from previous studies (Bloom, 1981; Hellou *et al.*, 1982; Thompson *et al.*, 1982) were recalculated for comparison with combined data for each species. Diet breadth was calculated using the Shannon diversity index (natural log) and niche overlap among diets was calculated using the simplified Morisita index (Krebs, 1998).

RESULTS

Sponge assemblage richness and diversity

Scott's Bay (SB) had almost one and a half times the number of sponge species of Seppings West (SW) and Dixon Reef (DXR) (Table 2). At all three sites one sponge species dominated the fauna in terms of both number of patches and area: *Antho (Acarnia) lambeii* at SB and *Cliona californiana* at DXR and SW (Table 2). Estimated curves for both richness and diversity plateaued close to observed values for DXR and SW, but not for SB, suggesting that additional species might be present at the latter site (Fig. 2). K-dominance curve slopes

Table 2. Sponge abundance and nudibranch prey use for dorid nudibranchs consuming spiculate demosponges at three sites in Barkley Sound

		DXR							SB							SW					
Species	#	Area	CL	DS	DM	DO	PN	#	Area	CL	DS	DM	DO	PN	#	Area	CL	DS	DO	PN	
Cliona californiana	HD	50	796	0	0	0	0	0	2	31	0	0	0	0	0	74	909	0	0	0	0
Suberites sp.	HD								1	36	0	0	0	0	0						
Weberella n. sp.	HD	2	44	0	0	0	0	0													
Halichondria panicea	HL								1	2	4	1	7	0	0	1	14	0	0	0	0
Hymeniacidon ungodon	HL	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	21	1
Haliclona (*Reniera*) sp.	HP	2	55	0	0	0	0	0	0	0	0	1	0	0	0						
Haliclona cf. *cinerea*	HP								0	0	0	2	0	0	0	0	0	0	1	0	0
Haliclona cf. *foraminosa*	HP								10	288	0	0	0	0	0	7	43	0	0	0	0
Haliclona gellindra	HP								1	12	0	0	0	0	0						
Neopetrosia vanilla	HP	2	11	0	1	0	0	0	1	13	0	2	0	0	0	0	0	0	2	0	0
Pachychalina sp.	HP	4	43	0	0	0	0	0													
Antho lambeii	P								50	296	0	0	0	0	0	27	65	0	0	0	0
Clathria pennata	P	2	25	0	0	0	0	0													
Hamacantha hyaloderma	P	5	61	3	0	0	0	6	1	36	8	1	0	0	8	1	3	3	0	0	8
Hymedesmia sp. A	P	0	0	1	0	0	0	0													
Isodictya rigida	P															0	0	0	2	0	0
Lissodendoryx firma	P	1	4	2	0	0	0	2	0	0	3	0	0	0	2	0	0	0	0	0	2
Mycale sp.	P	8	278	0	0	0	0	0	1	49	0	1	0	0	0	1	2	0	0	0	0
Myxilla incrustans	P	2	28	2	0	2	0	2	1	4	8	0	0	0	10	1	13	9	0	0	5
Plocamionida lyoni	P								0	0	5	0	0	0	3	0	0	0	0	0	5
Unknown diet									0	0	0	0	0	1	0	0	0	0	1	0	2
Unknown environment									9	65	0	0	0	0	0	2	9	0	0	0	0
Total		78	1345	8	1	2	1	10	78	832	28	8	7	3	23	114	1058	12	6	21	23
None				4	1	0	0	2			5	8	3	0	9			1	4	4	8
N				9	2	2	1	9			20	12	10	2	23			10	9	25	25
Multiple prey				2	0	0	0	2			9	2	0	0	6			3	1	0	5
S		10		4	1	1	1	3	15		5	6	1	2	4	9		2	4	1	6
H′ raw		1.37	1.34	1.32	0	0	0	0.95	1.48	1.77	1.54	1.73	0	0.69	1.21	1.04	0.62	0.59	1.33	0	1.59
H′ estimated		1.28	1.28						1.31	1.55						0.98	0.61				
Sp. max. abund.		64.1	59.2						64.1	35.6						64.9	85.9				
Sp. min. abund.		1.3	0.3						1.3	0.2						0.9	0.2				

Sites: DXR, Dixon Reef; SB, Scott's Bay; SW, Seppings Island, West (Fig. 1). # = number of patches observed, area in cm^2. Numbers in columns for nudibranchs represent isolations of each prey type; zeros are given only when the prey type was found in the environment. NONE = number of nudibranchs found with no spicules in faeces, *N* = total number of nudibranchs observed. *Doris montereyensis* was not found at SW. Sponge orders: HD = Hadromerida; HL = Halichondrida; HP = Haplosclerida; P = Poecilosclerida. Abundances and diversity calculated based on demosponge area. Multiple Prey = number of individuals with more than one prey item in faecal contents; S = species richness of diet; H′ = Shannon Diversity Index of diet, using natural logarithms; raw values directly calculated using raw abundance data; estimated values calculated using Estimate S. Sp. max. abund. = species of maximum abundance (percentage of total); Sp. min. abund. = species of minimum abundance. Dorid species: CL, *Cadlina luteomarginata* MacFarland, 1966; DS, *Diaulula sandiegensis* (Cooper, 1863); DM, *Doris montereyensis* Cooper, 1863; DO, *Doris odhneri* MacFarland, 1966; PN, *Peltodoris nobilis* (MacFarland, 1905).

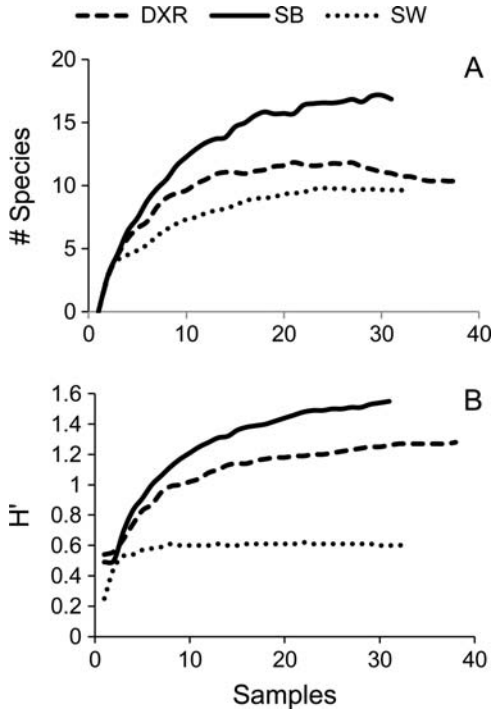

Figure 2. Cumulative species richness and diversity for field sites. Values estimated using EstimateS. **A.** Cumulative species richness. **B.** Cumulative diversity calculated using the Shannon index (H′-ln). Abbreviations: DXR, Dixon Reef; SB, Scott's Bay; SW, Seppings Island, West.

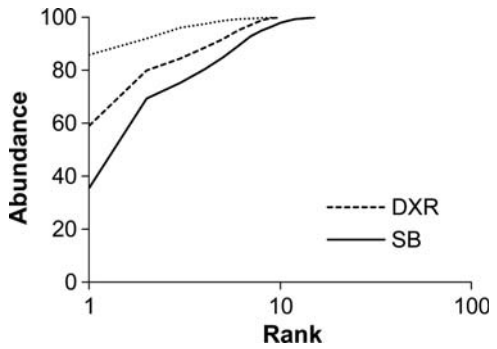

Figure 3. K-dominance curves of sponge fauna at field sites. Dominance curves calculated by area for each species. Abbreviations: DXR, Dixon Reef; SB, Scott's Bay; SW, Seppings Island, West.

(Fig. 3) show the relatively low diversity of all three communities, especially at SW.

Prey use by nudibranchs

Eight species of sponge-eating dorids were found at the three sites. *Cadlina luteomarginata* and *Peltodoris nobilis* were present at high numbers at all sites. *Diaulula sandiegensis*, *Doris montereyensis* and *D. odhneri* were only present in high numbers at one to two sites each. One to three individuals of *Cadlina modesta*, *Aldisa cooperi* and *A. sanguinea* were found over the three field sites, but their diets were not analysed. Diet breadths ranged from one to six prey species and diet diversity (Shannon index H') ranged from 0.59 to 1.73 across sites, but there was little correspondence with richness or diversity of the local sponge assemblage (Table 2). Species-accumulation curves for diet breadth approached an asymptote for *C. luteomarginata* and *P. nobilis*, but continued rising for *Diaulula sandiegensis* (Fig. 4), suggesting that the diet of the latter diet could be broader than that recorded. Although *Doris montereyensis* and *D. odhneri* generally consumed only one sponge species at each site (Table 2), whereas *D. odhneri* almost exclusively consumed *H. ungodon*, *D. montereyensis* consumed different sponges at each site. Diet diversity and evenness were only very broadly similar across geographic regions for the various species (± 0.3) and H' values for *D. odhneri* showed marked differences (Table 3).

The identity of sponges consumed varied little, with only one or two species changing among sites (Table 2). All species selected against *Haliclona* cf. *foraminosa*, *Cliona californiana*, *Mycale* sp. and *Antho (Acarnia) lambeii* (Table 2; Fig. 5). *Cadlina luteomarginata* and *P. nobilis* showed significant selection for several poecilosclerids. Unknown diet species at SW had spicules intermediate between *L. firma* and *M. incrustans*, but lacked sufficient microscleres for confident identification. *Cadlina luteomarginata* also selected *Halichondria panicea* at SB, but not at other sites. *Diaulula sandiegensis* selected at least one sponge from each order except Hadromerida. *Doris montereyensis* consumed only *H. panicea* at SB, but only *M. incrustans* at DXR where *H. panicea* was not found. *Doris odhneri* was nearly monophagous on *Hymeniacadon ungodon*. Several sponges were consumed in small quantities but were not significantly selected compared with their availability: *Mycale adhaerens* and *H. hyaloderma* by *Diaulula sandiegensis*; *Myxilla incrustans* by *D. montereyensis*; and *H. ungodon* by *P. nobilis* (Table 2). Individuals of *C. luteomarginata*, *Diaulula sandiegensis* and *P. nobilis* commonly had more than one prey species in their faeces, while *D. montereyensis* and *D. odhneri* never did (Table 2). Approximately a quarter of the dorids sampled had no recognizable prey in their faeces (Table 2).

The sponge species included in the diet of each dorid changed among geographic regions and studies, but showed broad patterns of use among sponge orders. Most sponges consumed were in the Halichondrida and Poecilosclerida; haplosclerid sponges were consumed in bulk only by *D. sandiegensis* and hadromerid sponges were consumed in generally small quantities by all dorids except *D. odhneri*. Only two out of the five dorids showed consistent focus on one sponge order across all studies: *D. montereyensis* consumed primarily halichondrid sponges, while *P. nobilis* consumed primarily poecilosclerids. The other three species shifted their use of sponge orders among sites. *Diaulula sandiegensis* had a broader diet at BS2 than at SJI. *Doris odhneri* and *C. luteomarginata* consumed a mix of halichondrid and poecilosclerid sponges in SCA and SJI, but consumed mostly poecilosclerid sponges at sites in British Columbia. For those studies that included more than one dorid species, niche overlap values were typically below 0.2, except for *C. luteomarginata* versus *D. montereyensis* and *P. nobilis*; the latter value was greater than 0.9 in the present study (Table 4). The pattern of among-species niche overlap values did not correspond with the generalizations of Bloom (1981).

DISCUSSION

Diets are not a simple function of the local sponge assemblage

These cryptobranch dorids are clearly not true generalist consumers of demosponges, as neither prey richness nor diversity matched site richness or diversity, and most of their prey items were significantly selected compared with their background abundance (Fig. 5). Diet richness and diversity increased with sponge assemblage richness and diversity for only two dorids (Table 2), while *P. nobilis* had the highest diet diversity at the site with the lowest sponge diversity. Despite differences in recorded prey, all dorid species have shown a similar range of

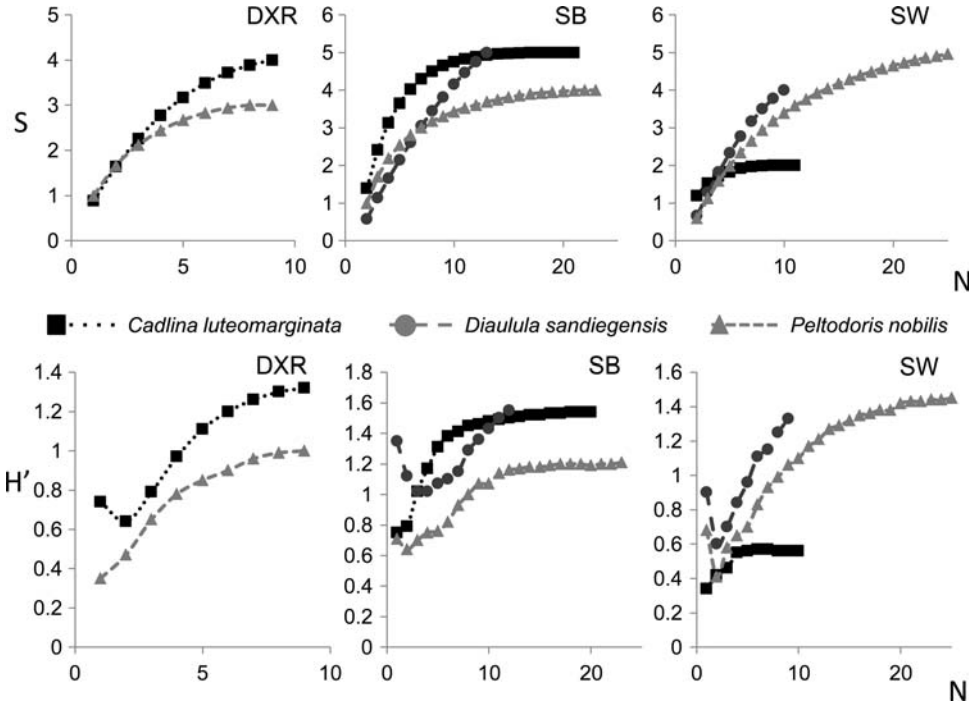

Figure 4. Accumulation curves for diet richness and diversity. S, species richness; H′, species diversity at three field sites. Abbreviations: DXR, Dixon Reef; SB, Scott's Bay; SW, Seppings Island, West.

diet diversity in previous studies (Table 3). Other studies have rarely included data on both diet and sponge assemblages, but the Mediterreanean *Platydoris argo* is known to select significantly among sponge species in a diverse sponge assemblage, showing diet breadth and diversity similar to those reported here (Megina *et al*., 2002). Sponge diversity in Barkley Sound is low compared with other studies of subtidal temperate reefs (Bell & Barnes, 2000; van Soest *et al*., 2007; Ruzika & Gleason, 2008; Berman & Bell, 2010) and is similar to values for McMurdo Sound, Antarctica (Dayton *et al*., 1974).

Dorids generally selected sponges that were uncommon to rare and avoided the most common species. In some other studies, dorids have primarily consumed the most conspicuous sponges (Dayton *et al*., 1974; Knowlton & Highsmith, 2000; Chu & Leys, 2012), although this not always the case (Megina *et al*., 2002; Gemballa & Schermutzki, 2004). Certain sponges were avoided by all five dorids: *Cliona californiana*, *Haliclona* cf. *foraminosa*, *Mycale* sp. and *Antho (Acarnia) lambeii* (Fig. 5). The reason for this is unclear, since *C. californiana* and *Mycale*, as well as other *Haliclona* species, are consumed by other dorid species (McDonald & Nybakken, 1997). An intriguing possibility is that the dorids are limiting their prey sponges to cryptic refugia in these habitats, as described for fish predators of Caribbean reef sponges (Pawlik, 1998). In communities where sponges are abundant, it is usually an assemblage, rather than one species, that dominates (Knowlton & Highsmith, 2000), yet all three sites were dominated by one sponge that was never consumed by dorids (Table 2). Exclusion experiments are required to determine whether dorid feeding limits sponge distribution, since the available evidence is equivocal (Dayton *et al*., 1974; Knowlton & Highsmith, 2000).

Roughly a quarter of dorids have empty guts at any time. Among species and sites, an average of 27% (range 0–66%) of individuals collected had no recognizable spicules in their faeces (Table 2), and similar values have been found for *C. luteomarginata* (Table 3), *Platydoris argo* and *Peltodoris atromaculata* in other studies (Megina *et al*., 2002; Gemballa & Schermutzki, 2004). Individual dorids might have trouble finding prey that are uncommon or inconspicuous. Alternatively, items besides spiculated demosponges may sometimes be eaten. Demosponges that lack spicules would not be detected in faecal analysis as used here, but are unlikely to affect the results significantly, because such species were not detected at these sites. Furthermore, *C. luteomarginata* is the only one of these dorids known to consume demosponges without spicules (McDonald & Nybakken, 1997) and is often noticeably purple when feeding on them (B. Penney, pers. obs.). Likewise, the comparable studies either reported gut contents (Megina *et al*., 2002) or also recorded what sponges the dorids used as substrate (Gemballa & Schermutzki, 2004) and would therefore have detected any unspiculated sponges. Dorids may also consume detritus, as previously found in *P. nobilis* (Kitting, 1981). Indeed, several of the 'empty' gut samples in this study contained diatoms or other particles found in detritus (barnacle exuvia, etc.) as also noted by others (Megina *et al*., 2002; Gemballa & Schermutzki, 2004).

Diet breadth and diversity show two distinct patterns

The five species of cryptobranch dorids sampled all had a specialized diet locally but a broader diet globally and therefore fit the definition of 'non-stereotyped specialist' *sensu* Megina *et al*. (2002). However, these species showed two separate patterns of diet breadth and diversity. Three species—*Cadlina luteomarginata*, *Diaulula sandiegensis* and *Peltodoris nobilis*—typically consumed two to six sponges belonging to several orders at each field site with high diet diversity (Table 2). All three species had many individuals (9–31%) whose faeces contained more than one prey item (Table 2). Based on diet accumulation curves (Fig. 4), *Diaulula sandiegensis* at these sites likely has a diet broader than that detected. All have even broader diets reported in the literature, with 20 or more prey species known (Bloom, 1981; McDonald & Nybakken, 1997; Chu & Leys, 2012). Richness and diversity for any given site were typically close to those for all three sites combined and also to results from other studies across a broader geographical range (Table 3). Diets among the three sites were broadly similar,

Table 3. Prey use and diet diversity compared across studies of dorid nudibranchs consuming spiculate demosponges in the northeastern Pacific

		CL					DS		DM		DO		GH	PN	
Order	Sponge species	SCA	SJI	BS1	BS2	HS	BS2	SJI	BS2	SJI	BS2	SJI	SJI	BS2	SJI
HD	*Suberites* sp.					10									
HD	*Terpios* sp.		3					2		4		27	5		1
HL	*Axinella* sp.	51													
HL	*Halichondria panicea*		15		8		7	35	78	76		37	14		5
HL	*Higginsia higginissima*	9													
HL	*Higginsia* sp.		35	4		7									
HL	*Hymeniacidon ungodon*										96			2	
HP	*Haliclona (Reniera)* sp.						7								
HP	*Haliclona* cf. *cinerea*						20	33		2		2	5		1
HP	*Neopetrosia vanilla*						33								
P	*Biemna rhadia*		1					1							26
P	*Desmacella sp*	2													
P	*Forcepia* sp. A	2													
P	*Forcepia* sp. B	2													
P	*Hamacantha hyaloderma*	2	8	15	29		7					5		39	16
P	*Hymedesmia* sp.	2		48	2	13									
P	*Hymenamphiastra* sp.	2													
P	*Isodictya rigida*						13								
P	*Leptolabis* sp.	2													
P	*Lissodendoryx firma*		5		10	3		1				4	5	11	3
P	*Mycale adhaerens*		5					8		4		5	19		14
P	*Mycale hispida*					3									
P	*Mycale lingua*		5					5				2	24		
P	*Mycale psila*		4							3		1	10		9
P	*Mycale richardsoni*														1
P	*Mycale* sp.						7								
P	*Myxilla incrustans*	26	18	33	40	63		16	22	9		17	19	30	25
P	*Plocamionida lyoni*				10									14	
	Unknown				2		7		0		4			4	
	Isolations	45		27	49	30	15		9		25			56	
	No. with empty gut	54		7	10	20	15		3		4			19	
	N	99	83	34	41	50	25	160	12	256	28	172	21	60	111
	S	10	10	4	7	6	8	8	3	6	2	9	8	6	10
	H'	1.115	1.899	1.123	1.554	1.194	1.859	1.529	0.53	0.928	0.168	1.664	1.91	1.437	1.529
	J'	0.484	0.825	0.810	0.799	0.666	0.894	0.735	0.482	0.518	0.242	0.757	0.919	0.802	0.664

Abbreviations: BS1, Barkley Sound, B.C. (Hellou *et al.*, 1982); BS2, Barkley Sound, B.C. (present study); HS, Howe Sound, B.C. (Hellou *et al.*, 1982); SCA, southern California (Thompson *et al.*, 1982); SJI, San Juan Island, Washington (Bloom, 1981); *N*, total number of nudibranchs examined; H' = Shannon diversity index of diet, using natural logarithms; J' = Shannon evenness. Numbers for each sponge species are percentage of total isolations from the nudibranch species studied. Dorid species: CL, *Cadlina luteomarginata*; DS, *Diaulula sandiegensis*; DM, *Doris montereyensis* 3; DO, *Doris odhneri*; GH, *Geitodoris heathi*; PN, *Peltodoris nobilis*.

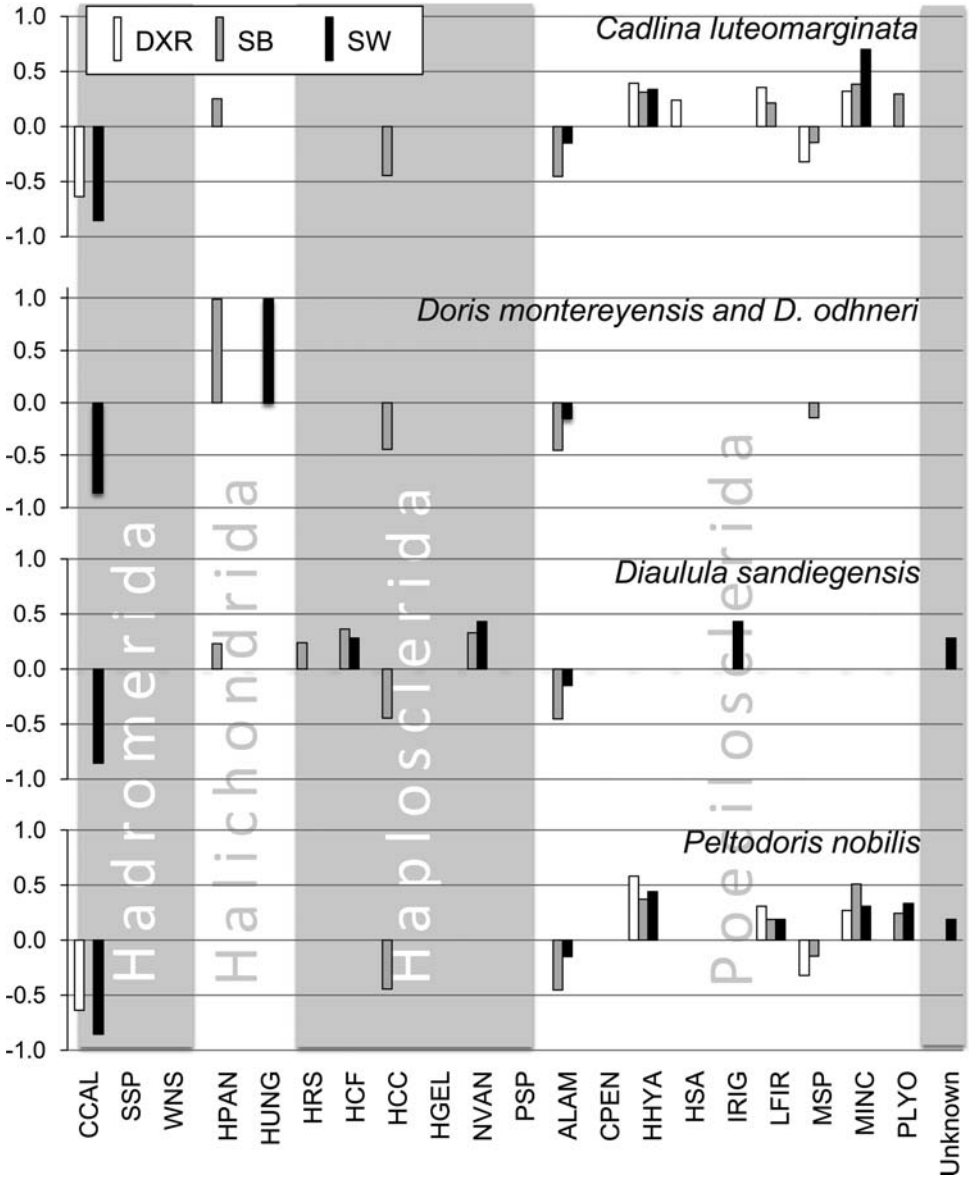

Figure 5. Selectivity of dorids for sponges in Barkley Sound, B.C. *X*-axis, sponge species (see Table 1). Shaded/unshaded background rectangles denote membership of sponge orders. All bars represent Pearre's *C* values, calculated based on percentage area versus percent usage as isolations. In all cases, open bars are from Dixon Reef (DXR), gray bars are from Scott's Bay (SB) and black bars are from Seppings West (SW). Only significant values ($P < 0.05$) are shown. Panels denote different dorid species, with *Doris montereyensis* at SB only and *D. odhneri* at SW only.

with niche overlap values among sites greater than 0.5 (Table 4). The percentages of each prey used varied among studies, even for the same species at the same location (e.g. *C. luteomarginata* at BS1 and BS2; Table 3). These species therefore all match the 'non-stereotyped specialist' profile of *P. argo* (Megina *et al*., 2002).

The two other species—*Doris montereyensis* and *D. odhneri*—typically consumed only one prey item at each site and individuals never had more than one prey item in their faeces (Table 2); all prey was significantly selected over background abundance (Fig. 5). *Doris odhneri* consumed *Hymeniacidon ungodon* almost exclusively, while *D. montereyensis* consumed *Myxilla incrustans* at DXR but *Halichondria panicea* at SB (Table 2). However, diets reported for these dorids range from 6 to 9 species in a geographic area (Table 3) and altogether over 17 species have been reported in the literature (Bloom, 1981; McDonald & Nybakken, 1997; Chu & Leys, 2012). The narrow diets could be an effect of small sample size for *D. montereyensis*, but this seems unlikely for *D. odhneri*. Local diets could be restricted due to ingestive conditioning to the most common prey item (Hall, Todd & Gordon 1982; Hall & Todd, 1984) or because these seemingly oligophagous species are comprised of stenophagous populations specializing on different prey (Todd *et al*., 2001; Thompson, 2005), as reported in the sacoglossan *Elysia viridis* (Sotka, 2005).

Diets of northeastern Pacific cryptobranch dorids may be generalized

While cryptobranch dorids have typically been described as being specialized on certain sponges possessing key attributes, the emerging picture is that at least some behave as generalized feeders on spiculate sponges. Data from other dorid species are sparse, but suggest that there may be a gradient of specificity among dorids as a whole. *Doris pseudoargus* has also been reported to be

Table 4. Niche overlap values for dorid nudibranchs

A.					
Species	DXR/SB	DXR/SW	SB/SW		
CL	0.808	0.633	0.689		
DS			0.537		
PN	0.808	0.802	0.887		
B.					
Species	CL	DS	DM	DO	
DS	0.120				
DM	0.322	0.129			
DO	0.001	0.005	0.000		
PN	0.949	0.129	0.142	0.035	
C.					
Species	CL	DS	DM	DO	GH
DS	0.452				
DM	0.266	0.679			
DO	0.473	0.679	0.741		
GH	0.470	0.575	0.362	0.561	
PN	0.422	0.331	0.179	0.374	0.523

A. Within species, among sites. B. This study, among species. C. Values calculated from Bloom (1981), among species. Values calculated as the simplified Morisita index. Dorid species: CL, *Cadlina luteomarginata*; DS, *Diaulula sandiegensis*; DM, *Doris montereyensis*; DO, *Doris odhneri*; GH, *Geitodoris heathi*; PN, *Peltodoris nobilis*. Sites: DXR, Dixon Reef; SB, Scott's Bay; SW, Seppings Island, West.

monophagous at some field sites (Thompson, 1964). *Peltodoris atromaculata* only feeds on *Petrosia* spp. and *Haliclona fulva* (Gemballa & Schermutzki, 2004) and chromodorids in general each feed exclusively on one or a few sponge species (Rudman & Bergquist, 2007). *Platydoris argo* is a 'non-stereotyped specialist', focusing on a few species at each site but changing the focal species among sites (Megina *et al*., 2002). *Doris kerguelensis* in McMurdo Sound shows a dietary richness and diversity similar to the more generalized Barkley Sound dorids (Dayton *et al*., 1974) and shifts prey use among sites (Barnes & Bullough, 1996). Of the cryptobranch dorids for which dietary information is available, roughly half are reported to be monophagous, but many of those species have only recently been described or are poorly studied (Todd *et al*., 2001). Indeed, even the current data are limited in space and time; observed diets may change with seasons or at additional field sites.

Bloom's taxonomic guild hypothesis (Bloom, 1976, 1981)—that cecate dorids consume sponges with disorganized skeletons while acecate dorids consume sponges with organized skeletons—was not well supported by the present results. Instead, all dorids investigated consumed sponges from multiple sponge orders with varying skeletal types. Both *P. nobilis* ('acecate') and *C. luteomarginata* ('cecate') fed largely on poecilosclerids (Table 2, Fig. 5) and show almost complete overlap in their niches (Table 4). Niche overlaps between 'cecate' and 'acecate' dorids were similar to overlaps between species in the same category (Table 4). Three of the five species shifted their use of halichondriid and poecilosclerid sponges among feeding studies (Table 3). One study did show *P. nobilis* consuming only poecilosclerids, but only studied 12 individuals (Kitting, 1981). Even though *D. montereyensis* and *D. odhneri* primarily consumed halichondriid sponges in the present study, both are known to consume demosponges from other orders (McDonald & Nybakken, 1997), and *D. odhneri* also consumes hexactinellids (Chu & Leys, 2012). *Cadlina luteomarginata* also consumes keratose sponges that lack spicules altogether (Cattaneo-Vietti & Balduzzi, 1991; McDonald & Nybakken, 1997), although their relative importance in the diet is not clear. Prey skeletal organization does not appear to be a major determinant of prey selection in these dorids.

Likewise, the view that diets of cryptobranch dorids are constrained by the need for chemical defence (Faulkner & Ghiselin, 1983; Cimino & Ghiselin, 1999) does not appear to hold here. All five of the species investigated biosynthesize defensive compounds (Avila, 1995; Kubanek & Andersen, 1999) and *C. luteomarginata* may be able either to biosynthesize or to sequester its defensive compounds, depending on availability from prey (Kubanek, Faulkner & Andersen, 2000). Dorids may also be limited by their ability to detoxify prey defences, as known in other taxa (Sotka *et al*., 2009; Whalen *et al*., 2010). However, the suite of sponges consumed by multiple dorids over multiple field sites (Table 3) suggests that some sponges may be broadly palatable. Without the need to obtain chemical defences, diet breadth may be a balance between avoiding prey with potent defences while accepting a broad enough spectrum to overcome prey patchiness (Hughes, 1980; Bloom, 1981). An interesting possibility is that species that biosynthesize their chemical defences have broader diets than those that sequester them. Of the other species for which quantitative dietary studies are available, *D. macmurdensis* may biosynthesize its defences, *P. atromaculata* sequesters them from its prey, but the strategy of *P. argo* is not known (Avila, 1995; Goclik, König & Wright, 1999). The relative frequency of broad versus narrow diets should be further clarified before accepting theories ascribing the evolution of prey use to any particular factor.

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