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Using polyclonal antibodies to detect predation by dogwhelks *Nucella lapillus* (L.)

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ABSTRACT

Interactions between predators and their multiple prey species can vary greatly among locations where they coexist. As a method to assess spatial variation in predation by intertidal dogwhelks on their dominant prey, immunoassays of dogwhelk gut contents from experimental populations and field collected individuals were evaluated using polyclonal antibodies raised separately to soluble proteins from *Mytilus edulis* L. mussels and *Semibalanus balanoides* (L.) barnacles. Both antisera produced strong reactions against their homologous antigens but no cross reactions between prey species. Experimental trials tested the critical hypothesis that prey species had equal detection intervals in dogwhelk guts. Two groups of 225 dogwhelks were starved for 14 days, provided with either mussels or barnacles for five days, and then sampled over 22 days. Independent immunoassays of dogwhelk gut contents against each antibody revealed a consistent, weak cross reaction between the anti-mussel antibody and dogwhelk gut tissues. After accounting for this cross reaction, the strength of immunoassays against both prey species declined exponentially and at similar rates. The proportions of dogwhelks that tested positive for their provided prey species declined linearly through time and were not significantly influenced by prey type. Prey were detectable throughout the sampled post-feeding period and were projected to have detection limits of 24.4 days (barnacles) and 26.5 days (mussels), demonstrating that immunoassay results are not biased by dissimilar prey detection intervals. Reactions against the antibody from the non-provided prey were time invariant and occurred at relatively low frequencies. Immunoassays of dogwhelks collected from five intertidal sites on Swans Island, Maine, USA revealed patterns similar to field observations, though immunoassays classified far fewer individuals as non-feeders and more as barnacle feeders than indicated by direct field observations. Unlike single observations, immunoassays also revealed the presence of both prey in dogwhelks from four sites, though most individuals tested positive for only a single prey type. Immunoassays facilitate concurrent collections of predation data from many individuals and will enable further local- to regional-scale assessments of dogwhelk predation at additional sites around the Gulf of Maine.

1. Introduction

Accurate empirical determinations of where and when who eats whom remain central to tests of any ecological theory involving predation. In their simplest form, data on predator-prey interactions provide information on trophic connections and food web topologies, which can differ markedly among sites that contain similar assemblages (Menge and Sutherland, 1976; Paine, 1980; Leonard et al., 1998; Sanford et al., 2003; Polis et al., 2004). One reason for spatial variation in predator-prey interactions is differences in the prey community, as the presence of alternative prey can alter the timing and magnitude of predation (Fairweather, 1985; Petraitis, 1998; Wieters and Navarrete, 1998; Berlow, 1999; Sanford et al., 2003). There is currently much interest in trophic interactions beyond analyses of who eats whom to quantify variable consumption rates (e.g., Berlow et al., 2004). An understanding of when and where predators and prey interact across large spatial scales, however, can also provide much information on the variable influence of predators (Foster, 1990; Sanford et al., 2003). Such analyses depend on reliably detecting predation.

Detecting predation has been advanced through the application of molecular methods to identify cryptic prey, even in minimal gut contents (Greenstone, 1996; Symondson, 2002; Sheppard and Harwood, 2005). These techniques (e.g., polyclonal antibodies, monoclonal antibodies, stable isotopes, and a host of DNA analyses within the last decade) share the advantage of facilitating the collection of simultaneous, large spatial scale data for later laboratory analyses. Such data are often not easily obtained using either experiments at multiple locations or repeated observations of free-ranging animals. They also allow sampling of individuals that had exhibited natural behaviors in natural prey assemblages, thereby eliminating potential experimental artifacts. The application of each molecular method to

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ecological questions, however, is constrained by limits of their specificity and sensitivity to prey, as well as their capacity to provide quantitative, as well as qualitative data (Feller and Ferguson, 1988; Sopp et al., 1992; Sunderland, 1996; Naranjo and Hagler, 2001; Symondson, 2002; Sheppard and Harwood, 2005).

Polyclonal antibody methods (hereafter 'immunoassays') have long been successfully applied in parallel in entomological studies of predators that consume soft tissues (Greenstone, 1996; Sunderland, 1996), and in marine ecosystems to determine food web interactions that cannot be easily observed *in situ* (Feller et al., 1979, 1985; Hunter and Feller, 1987; Feller, 1984; Scholz et al., 1991; Taylor, 2004; but see Mayfield et al., 2000). Immunoassays incorporating precipitin reactions can potentially provide inexpensive assessments of predation (Feller et al., 1979), though these methods have largely been eclipsed by much more sensitive molecular methods developed specifically to reduce the potentially confounding cross reactions with non-target prey that are common to polyclonal antibody methods (Greenstone, 1996; Symondson, 2002; Sheppard and Harwood, 2005). Polyclonal antibody methods also generally detect prey over longer periods than either monoclonal antibody methods or DNA analyses (Symondson, 2002). This characteristic could be an advantage or disadvantage depending on the study system and research question. However, like many other methods immunoassays do not reveal how many prey were consumed without incorporating additional estimates of how many prey are present per positive reaction (Feller and Ferguson, 1988; Sopp et al., 1992; Sunderland, 1996; Naranjo and Hagler, 2001). These limitations—the greatest being the potential for cross reactivity with non-target prey—suggest that polyclonal antibodies remain an appropriate method for inexpensively identifying distantly related prey within predators already known to specialize on few prey species.

Nucella lapillus (L.) dogwhelks occur in the intertidal zone on rocky shores on both sides of the north Atlantic and have been reported to prey primarily on *Mytilus edulis* L. mussels and *Semibalanus balanoides* (L.) barnacles (Connell, 1961; Largen, 1967; Menge, 1976; Crothers, 1985; Hughes and Drewett, 1985; Petraitis, 1998; Fisher, 2005). For example, Menge (1976) reported that 99.5% of n=1265 dogwhelks sampled at five sites within three years in Maine and Massachusetts, USA were observed feeding on mussels or barnacles. This pattern of predation on the two dominant sessile animals persists even though they occasionally consume a wider range of prey at certain locations (Menge, 1976; Hughes and Dunkin, 1984; Crothers, 1985). Given the apparent dominance of mussels and barnacles in dogwhelk diets, such a pattern of predation is conducive to immunoassay tests on individuals, as only two tests per dogwhelk must be performed. Such a small number of tests allow investigations beyond what the species eats to address when and where individuals consume one or both prey types. This contrasts with the large number of tests and pooling of small-bodied predators necessary when many prey may be consumed, as individual analysis of a small predator's gut is precluded when many species are potential prey since there are not enough gut contents to enable tests against all antisera (e.g., Feller, 1984).

Here I describe the production, specificity, and temporal detection limits of prey antigens using polyclonal antibodies raised separately to soluble proteins from barnacle and mussel tissues. I further evaluate the strengths and limitations of using immunoassays rather than single observations to resolve patterns of dogwhelk predation at five intertidal sites on Swans Island, Maine, USA where mussels and barnacles are the dominant sessile animals. With an understanding of the specificity and temporal patterns of immunoassay reactions derived from the experiments, polyclonal antibodies can successfully discriminate between the presence of one or both prey from field collections of predators at intermediate spatial scales (1–15 km around Swans Island). These immunoassays will therefore facilitate future evaluations of the dependence of dogwhelk predation on the local distribution and abundance of its two dominant prey species at a total of 19 sites and at spatial scales of up to 400 km around the Gulf of Maine. Those analyses will be presented in a separate publication.

2. Methods

2.1. Prey species antigen preparation

Mussels and barnacle-covered cobbles were collected in May 2004 from Swans Island, Maine, USA. Prior to antigen preparation, both species were isolated in aerated seawater tanks for five days (water changed daily) to rid their guts of foreign materials.

To make antigens, dissected tissues from approximately 200 barnacles (opercula diameters 2–6 mm) and from 20 mussels (15–35 mm shell lengths) were homogenized separately using a chilled mortar and pestle in a minimum (~1 mL) of TES-saline buffer: 5 mM TES (N-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid); 150 mM NaCl; concentrated NaOH raised the pH to 7.3. Homogenates were centrifuged for 10 minutes at 15100×*g* under refrigeration. Centrifugation was repeated until minimal pellets formed and the supernatant liquids cleared. Protein concentrations of the supernatant antigens were quantified against a known bovine serum albumin dilution curve (Bradford, 1976). Antigens were diluted in TES-saline buffer and the final concentration of the barnacle antigen was 2.3 mg mL^{-1}, while the mussel antigen was 3.9 mg mL^{-1}. Both antigens were stored at −20 °C and provided for antibody production at these concentrations. Polyclonal antibody production was performed by Cocalico Biologicals, Inc. (Reamstown, Pennsylvania, USA). Both the antibody-antigen prescreening tests and 37 day immunization schedule for two rabbits per antigen followed the protocols outlined by Feller et al. (1979).

2.2. Double-diffusion immunoassay tests

Screening of pre- and post-immunization rabbit sera, as well as dogwhelk gut content analyses were all performed using double-diffusion immunoassay tests in agarose gel (Öuchterlony, 1968; Feller et al., 1979). All individual gut contents were drawn from the stomachs of thawed dogwhelks using a Pasteur pipette attached to a syringe and contents were diluted in a single drop (~25 μL) of TES-saline buffer solution before being immediately refrozen in microcentrifuge tubes on dry ice. While gut volumes from individual dogwhelks differed by up to a factor of five (10–50 μL), antigens used to produce the antisera reacted against their homologous antiserum at antigen dilutions (in TES-saline buffer) of at least 1:100. Therefore, given the high sensitivity of the tests to detect antigens and dilution of gut contents in a constant volume, no individual was excluded from testing due to having a gut volume <50 μL as this may have biased against the detection of true non-feeding individuals. Dogwhelk stomachs are relatively simple, having lost many of the structural complexities seen in other mollusk species (Graham, 1949), and both their location and contents are visible when the shell is carefully cracked using a vice. These two features of dogwhelk anatomy facilitated gut content sampling.

Immunoassays were performed on 25×75 mm microscope slides covered with a 0.5% analytical agarose gel under plastic antibody-antigen well templates using the methods described by Feller et al. (1979), with the following modification. In place of Veronal and sodium Veronal in the analytical gel recipe (Feller et al., 1979), 0.05 M Tris hydrochloride and 0.05 M Tris base were substituted (concentrated NaOH raised the pH to 8.6).

Reactions on each slide involved placing 10 μL of antiserum into the central well of a template and 10 μL of centrifuged supernate (2000×*g*, 1 min) from four samples was added to individual wells around each central well (Fig. 1). For gut analyses, four dogwhelks' gut contents were tested against both antisera using two five-well templates on a single slide. Double diffusion reactions proceeded at room temperature in humidified chambers for 48 hours. Gels were then rinsed, dried and stained using protocols described by Feller et al. (1979).

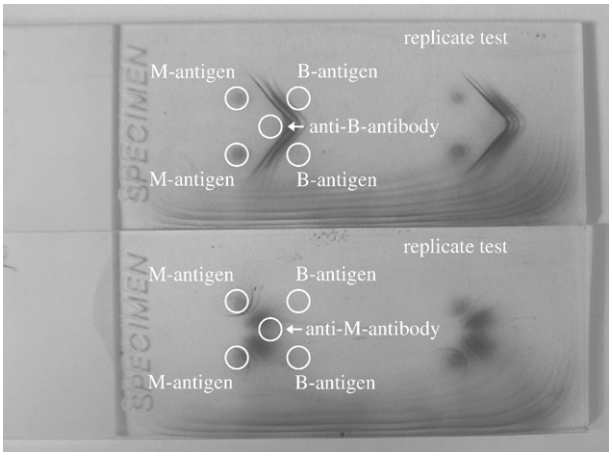

Fig. 1. Results of double diffusion polyclonal antibody-antigen tests in agarose gel on microscope slides. Locations where antigen extracts from mussels (M) and barnacles (B) and the antibodies were placed are indicated by white circles (plastic well templates removed). Stained lines between adjacent antibody and antigen wells indicate precipitin reactions. The top slide shows replicated reactions involving anti-barnacle antibody; the bottom slide shows reactions involving anti-mussel antibody. Faint horizontal lines at the bottom of both slides are due to agarose drying.

After reactions were complete, the stained slides show antigen–antibody precipitin lines between adjacent wells wherever reactions occurred (Fig. 1). The resulting precipitin lines were counted using a dissecting microscope at 20× magnification and incident light. Mussel and barnacle antigens formed precipitin reactions against their homologous antiserum when antisera were diluted up to one hundred fold using distilled water. Both antisera titres were therefore 1:100.

2.3. Prey antigen detection through time

In order to determine whether the barnacle and mussel detection periods were similar for each antibody, dogwhelk feeding trials were performed in the laboratory. During a single low tide, 450 dogwhelks (size range 20.58–36.97 mm shell lengths) were collected from Pemaquid Point, Maine (43°50.15′ N; 69°30.45′ W) in July 2006. This is a site where dogwhelks had previously been observed feeding on both mussels and barnacles and individuals tested positive for either or both prey in immunoassay tests (J.A.D. Fisher, *unpublished results*). At the Darling Marine Center, Walpole, Maine dogwhelks were randomly and equally divided into two flow-through seawater tanks (n=225 per tank, tank dimensions approximately 0.9 m×0.9 m×0.7 m deep) at ambient temperature. Tanks were covered with screens at the water line to keep the dogwhelks submerged at equal temperatures to minimize potential differences in digestive rates between dogwhelks in or out of the water.

The following experimental starvation, feeding and sampling protocol was performed. Dogwhelks were first starved for 14 days. During the starvation period, the tanks were rinsed out at least once every 48 hours to remove sediment and the few dogwhelks that died during the starvation period (8 died in the barnacle tank; 6 died in the mussel tank). On day 14, 26 barnacle-covered cobbles were added to one tank and >500 mussels were added to the other tank. Mussel lengths (most 15–30 mm) and barnacle opercula diameters (>1.5 mm) were within the size ranges selected preferentially by similar sized dogwhelks (Bayne and Scullard, 1978; Hughes and Drewett, 1985). Dogwhelks were allowed to feed for five days, during which the tanks were not rinsed. This feeding duration was chosen to account for the observations that dogwhelks often require days to handle and ingest mussels of this size and that cumulative mussel consumption increases during the first four days of feeding after starvation (Bayne and Scullard, 1978; Hughes and Dunkin, 1984; Rovero et al., 1999). Though times for dogwhelks to handle and ingest barnacles are generally shorter than for mussels, dogwhelks consume similar amounts of either prey per unit time (Hughes and Drewett, 1985).

After five days, the remaining prey were removed, the tanks were rinsed out and 15 dogwhelks from each tank were randomly sampled and immediately frozen at −20 °C ('day 0 post-feeding'). Individual samples were labeled using unique, randomly assigned numbers to conceal the sample date and prey treatment during laboratory analyses. The same rinsing and sampling procedures were repeated daily for four days, then every two days for 18 additional days. This 22 day schedule was chosen in an attempt to exceed the maximum duration of prey detection and no dogwhelks died during this post-feeding period. Immunoassay tests against both potential prey from individual gut contents were used to determine the strength of reactions (number of precipitin lines) against both the prey species provided and the species not provided, as well as the temporal limits of positive reactions. Confidence intervals for proportions were calculated using the score method with continuity correction (Newcombe, 1998).

Since polyclonal antibodies often cross react with species other than the target prey due to shared antigens (Feller et al., 1979; Greenstone, 1996), the specificity of each antiserum was initially evaluated by testing each prey's antigens against each antiserum (Fig. 1). Additionally, potential cross reactions between dogwhelk tissues and prey-derived antisera were evaluated using two methods. The first method was inferential and based on the numbers of precipitin lines formed on each sampling date throughout the experimental period. If no cross reaction occurred between dogwhelk tissues and each antiserum, the numbers of precipitin lines should have declined towards zero during the post-feeding period. When >0 precipitin lines persisted through time, this pattern suggested a cross reaction between dogwhelk antigens and anti-prey antiserum. The second method directly evaluated these potential cross reactions using 'identity reactions' (Öuchterlony, 1968; Hunter and Feller, 1987). When cross reactions were inferred, immunoassays were performed using one anti-prey antiserum in the central well and homologous prey antigen alternating with antigen from dogwhelks (whose stomachs and digestive tracts had been removed) in the surrounding four wells. These tests eliminated potentially cross reacting antigens within dogwhelk guts. Therefore, if the anti-prey antiserum reacted with the same antigens from the dogwhelk tissue and the antiserum's homologous antigen, some precipitin lines would be continuous between adjacent wells around the central well. These identity lines can be quantified and provide strong evidence of the number of antigenic determinants shared between predator and prey (Hunter and Feller, 1987).

2.4. Field collections

With the goal of comparing patterns of dogwhelk predation based on field observations to immunoassay results, 19 to 25 dogwhelks per site were collected from the mid intertidal zone (approximately +0.6 m mean low water) at five locations on Swans Island, Maine in June 2004 (Table 1). Distances between sites ranged from 1 to 15 km. After randomly selecting individuals >18 mm shell length within haphazardly placed 0.25 m^2 quadrats, each dogwhelk was slowly turned over, observed, and recorded as feeding on mussels or barnacles, or not feeding (Connell, 1961). Dogwhelks were collected, individually labeled, frozen, and stored at −20 °C. Immunoassays were

Table 1
Site names, geographic coordinates, and site IDs (used in Fig. 5) of locations on Swans Island, Maine, USA where *Nucella lapillus* was collected

Site Name	Geographic Coordinates	Site ID
Basil	44°10.44′ N; 68°25.38′ W	BL
Pole 24	44°09.62′ N; 68°28.82′ W	P24
July 4th	44°09.31′ N; 68°28.25′ W	J4
Mill Pond	44°08.44′ N; 68°26.53′ W	MP
Ledges	44°08.14′ N; 68°27.15′ W	LD

performed as described above and individual gut contents were classified as positive for barnacles only, positive for mussels only, positive for neither prey species, or positive for both prey species.

3. Results

3.1. Qualitative immunoassay patterns

Double diffusion immunoassays revealed no reactions between prey antigens and pre-immunization rabbit sera. Post-immunization tests revealed consistent, positive reactions between homologous antibodies and antigens, with no cross reactions between prey types (Fig. 1). Both species' homologous reactions yielded consistently stronger results from one of each pair of immunized rabbits (mussel: maximum 5 precipitin lines; barnacle: maximum 6 precipitin lines). Therefore, antisera from the paired immunized rabbits were not pooled and all tests were performed using antiserum from one barnacle-immunized rabbit and one mussel-immunized rabbit. Each rabbit produced >50 mL of antiserum, which could provide up to 20000 tests of dogwhelk guts against each antibody.

Initial examinations of dogwhelk gut contents suggested a weak cross reaction occurred between dogwhelk tissues and the anti-mussel antibody. For example, 96% of the dogwhelk guts tested from field collected individuals formed at least one precipitin line with the anti-mussel antibody (maximum 5 precipitin lines), even at locations where mussels were extremely rare. No such cross reaction was evident for tests of dogwhelk gut contents against the anti-barnacle antibody.

3.2. Immunoassay strengths and temporal detection limits

Throughout the five day feeding interval, approximately 90% of dogwhelks were observed at the bottoms of the tanks handling and consuming prey. These observations suggest that dogwhelks did not stop feeding during this period. Before and after the feeding interval, however, approximately 70% of the dogwhelks were observed on the surface screens. Following the feed-starve experiment, the average numbers of precipitin lines showed significant non-linear declines through time following feeding (Fig. 2). Exponential fits based on the homologous reactions showed similar declines through time (half lives of 4.8 and 6.6 days for mussel and barnacle reactions, respectively) but had very different intercepts due to a consistent two-line cross reaction between dogwhelks and the anti-mussel antibody (Table 2). This cross-reaction is evident 22 days post-feeding, as the number of barnacle precipitin lines predicted by the regression approaches zero and the predicted number of mussel precipitin lines approaches the specified two line asymptote (Fig. 2). Of the dogwhelks fed mussels and tested against the anti-mussel antibody, 99% of immunoassays displayed at least one precipitin line, while 83% revealed two or more precipitin lines. Further support for the dogwhelk-mussel cross reaction comes from gut contents from barnacle-fed dogwhelks tested against the anti-mussel antibody. Barnacle-fed dogwhelks reacted against anti-mussel antibody in 98% of the tests (at least one precipitin line, n=207), with 56% having at least two precipitin lines. Finally, tests involving 'identity reactions' resolved the number of antigens shared between dogwhelks and mussels. A maximum of two lines of identity were formed in tests involving adjacent mussel- and dogwhelk-derived antigens. This pattern indicated two shared antigens from dogwhelks and mussels reacted against the anti-mussel antibody, thereby confirming the two precipitin line cross reaction (Fig. 2).

Table 2
Exponential regressions ($f=y_0+a*\exp(-b*t)$) between the average numbers of precipitin lines ('Lines') as functions of days since feeding (t) for dogwhelks fed mussels and barnacles

Feeding Treatment	Intercept	Slope	R^2	*F*-ratio	df	*P*-value
Mussel-Lines	3.29	$y_0=2$ $a=1.29$ $b=0.143$	0.65	10.41	2, 11	0.003
Barnacle-Lines	1.23	$y_0=0$ $a=1.23$ $b=0.105$	0.53	13.54	1, 12	0.003
Mussel-Logit	1.05	−0.14	0.78	41.93	1, 12	<0.0001
Barnacle-Logit	0.44	−0.12	0.42	8.84	1, 12	0.012

These regressions were calculated using methods for more than one dependent value for each independent value (Sokal and Rohlf, 1995; pp. 476). Linear regressions between Logit-transformed proportions ($\text{Logit}=\ln[p/q]$; where p=proportion, $q=1-p$) of dogwhelks ('Logit') that tested positive for their mussel or barnacle prey as a function of days since feeding.

After subtracting two precipitin lines from each test involving anti-mussel antibody, the proportions of dogwhelks that tested positive for the two prey species also declined during the post-feeding period (Fig. 3). Regressions on logit-transformed proportions showed that these functions declined linearly and at similar rates (Table 2), and the slopes of these regression lines did not differ significantly between treatments ($P=0.68$). ANCOVA further revealed that though the logit-

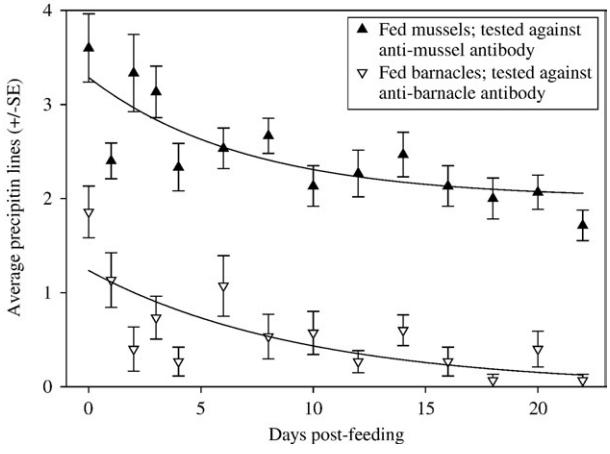

Fig. 2. Average numbers of precipitin lines (+/− SE) formed per immunoassay test as a function of days since the end of the experimental feeding period (where 0 days post feeding represents the end of the fifth feeding day) for dogwhelks fed mussels (filled symbols) or fed barnacles (open symbols) then tested against the prey's homologous antibody. Fitted exponential regressions from Table 2 are shown. Averages are based on samples of 14 or 15 dogwhelks.

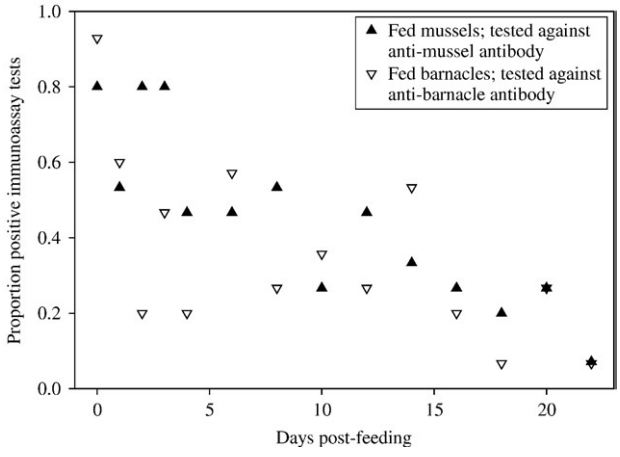

Fig. 3. Proportions of dogwhelks that tested positive for prey antigens as functions of days since the end of the experimental feeding period for dogwhelks fed mussels (filled symbols) or fed barnacles (open symbols) then tested against the prey's homologous antibody. Positive anti-mussel antibody proportions included only those tests displaying >2 precipitin lines (see text). Proportions are based on samples of 14 or 15 dogwhelks.

transformed proportions positive were influenced by days since feeding (Table 2), these proportions were not significantly influenced by prey species (P=0.17). Based on back-calculated proportions and the assumption of continued linear decreases in proportions positive through time, barnacles would be detectable for 24.4 days post-feeding, while mussels would be detectable for 26.5 days post-feeding.

3.3. Long term reactions

Since dogwhelk gut contents were tested against both antibodies following the five day experimental feeding interval and dogwhelks had been held apart from one prey species for a minimum of 19 days before sampling, these tests had the capacity to reveal long-term prey antigen persistence. These tests did not reveal significant temporal trends (Fig. 4). Mean proportions positive, however, were significantly higher in dogwhelks fed mussels and tested against anti-barnacle antibody (0.13; 95% CI 0.09–0.18) than in dogwhelks fed barnacles and tested against anti-mussel antibody (0.03; 95% CI 0.01–0.07) (Fig. 4).

3.4. Field collections

Field collected dogwhelks showed high among-site variation in both observations of predation and immunoassay results (Fig. 5). Smaller sample sizes for immunoassays relative to field observations at all sites (numbers above bars in Fig. 5) are due to dissection difficulties encountered with these earliest samples and the rare presence of parasites that obscured the gut location. Viable immunoassay tests, however, were obtained from >90% of individuals collected (Fig. 5). Overall, non-feeding dogwhelks were most frequently observed (50% of total individuals; range 32% to 74% among sites). Barnacle feeders were most frequently observed at BL and P24 sites (Table 1), while only at J4 were mussel feeders most frequently observed (Fig. 5).

Immunoassay results from these same individuals showed similarities and some contrasts to field observations. Within four sites (BL, LD, MP, P24), immunoassay results indicated that the majority of dogwhelks (61% to 82%) contained barnacle antigens only. At J4 most individuals (58%) tested positive for mussels only, which paralleled field observations. Associated with these immunoassay results, relative to field observations of non-feeders there was a marked decrease in the frequency of dogwhelks classified as positive for neither prey via immunoassays (22% of total individuals, range 9% to 33%

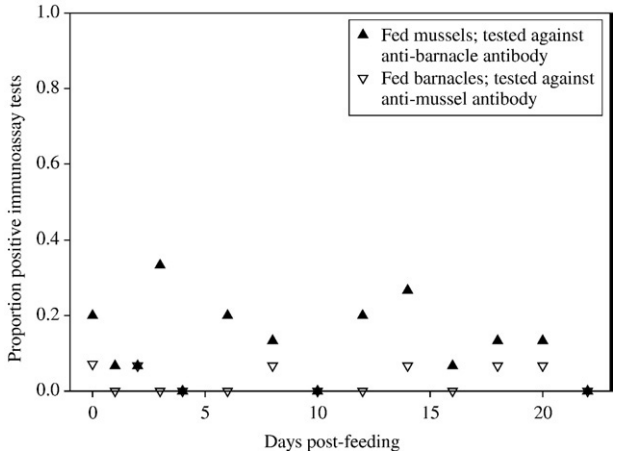

Fig. 4. Proportions of dogwhelks that tested positive for prey antigens as functions of days since the end of the experimental feeding period for dogwhelks fed mussels (filled symbols) or fed barnacles (open symbols) then tested against the non-provided prey's antibody. Positive anti-mussel antibody proportions included only those tests displaying >2 precipitin lines (see text). Proportions are based on samples of 14 or 15 dogwhelks.

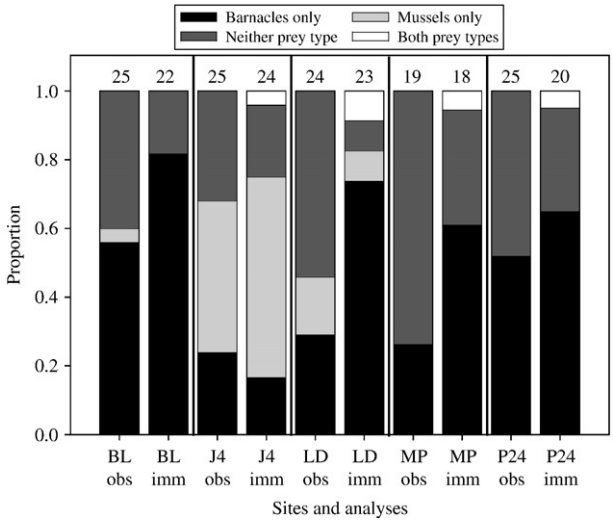

Fig. 5. Results from dogwhelks sampled at five sites (Table 1). Bars labeled 'obs' display the proportions of dogwhelks observed feeding on barnacles or mussels or not feeding when collected. Bars labeled 'imm' show proportions of those collected dogwhelks that tested positive for mussels only, barnacles only, for neither prey, or positive for both prey via immunoassays. Proportions classified as positive when tested against the anti-mussel antibody included only those tests displaying >2 precipitin lines (see text). Numbers above each bar indicate sample sizes.

among sites). Many of the observed non-feeders tested positive for barnacles, which dominated immunoassay results at four sites (Fig. 5). Overall, 64% of dogwhelks tested positive against barnacle antiserum, while only 38% were observed feeding on barnacles. In contrast, the overall percentage of individuals that tested positive against mussel antiserum (20%) was similar to the percentage observed feeding on mussels (14%). Finally, within these overall immunoassay percentages, dogwhelks tested positive for both prey species at four sites in frequencies that ranged from 4% to 9%. This fourth category of predation was exclusive to immunoassays, as single field observations could not reveal predation on more than one prey type.

4. Discussion

Both the importance and challenge of identifying and quantifying field collected marine invertebrates' gut contents have been recognized for more than 95 years, as highlighted by Petersen and Jensen's (1911) finding:

> "In the animal community the Gastropods and *Asterias* must be regarded as predatory animals; but though it may be very easy to capture the whelks on pieces of cod, (*Buccinum* and *Nassa*), it is just as difficult on the other hand to ascertain what they eat under normal conditions; their stomachs are almost always empty or contain a slime, the origin of which it has not been possible to determine."

Visual analysis of dogwhelk gut contents from the experimental trials provided equally vague information. Only two of 416 guts had identifiable prey remains (barnacle cirri within barnacle-fed dogwhelks on day 0 post-feeding). In contrast to observations, polyclonal antibodies provide a potential molecular technique to identify the presence of prey, though antigen detection duration and the extent of expected cross reactivity inherent to polyclonal antisera must be evaluated experimentally (Feller et al., 1979; Feller and Ferguson, 1988).

Among many tests using these antibodies, heterologous antisera and antigens from mussels and barnacles do not form precipitin lines and comparisons between homologous reactions are qualitatively different (Fig. 1). Homologous mussel reactions tend to produce many,

thin precipitin lines, while barnacle reactions produce fewer, thick precipitin lines. The cause of these differences in numbers of precipitin lines has now been identified.

If the number of precipitin lines is viewed as representing the 'strength' of immunoassays, then it originally appeared that mussel immunoassays reacted stronger than barnacle tests (Fig. 1). This difference, however, is due to a consistent cross reaction between dogwhelks and mussels, which are both mollusks. Though the strength of reactions against mussel- and barnacle-derived polyclonal antibodies decline at similar rates (Fig. 2), the number of precipitin lines differ consistently by an average of two (Table 2; Fig. 2) and identity reactions confirmed the presence of two shared, reactive antigens between dogwhelks and mussels. Knowing the magnitude of this cross reaction between predator and prey greatly improves the accuracy of immunoassay interpretations from field collected dogwhelks, as underestimates or overestimates of the cross reaction would artificially inflate or reduce the numbers of dogwhelks scored as mussel feeders, respectively.

After removing the two cross reacting precipitin lines, the proportions from experimental trials showing positive immunoassay tests for both prey species begin at similar levels and decline at rates that are not significantly different (Fig. 3), leading to very similar estimated maximum prey detection intervals. These similarities occurred despite known differences in the range of dogwhelk handling times for mussels (12–150 hours; Largen, 1967; Bayne and Scullard, 1978; Hughes and Dunkin, 1984; Rovero et al., 1999) and barnacles (3–24 hours; Connell, 1961; Largen, 1967; Dunkin and Hughes, 1984). These prey detection intervals are also much longer (weeks vs. hours) than those estimated for other small marine predators via immunoassays (e.g., Scholz et al., 1991; Taylor, 2004).

Prey-specific detection intervals of dogwhelks held in the laboratory probably differ from the field collected dogwhelks because of variation in water and air temperatures, submergence time, and behavior. Largen (1967) documented linear increases in dogwhelk consumption of mussels and barnacles with increasing temperature in the laboratory, while Bayne and Scullard (1978) demonstrated that the duration of non-feeding periods was reduced at higher water temperatures and that increasing dogwhelk starvation period increased subsequent mussel consumption. In the current trials, the timing of starvation and feeding coincided with the time of maximum average summer seawater temperatures across the Gulf of Maine (Fisher, 2007). Therefore, laboratory feeding rates were probably maximized due to warm water conditions and starvation. Dogwhelks also were kept submerged for the duration of the experiment in order to minimize differences in digestive rates resulting from different body temperatures. In addition to decreasing the variance in digestive rates, this treatment probably reduced the average digestive rate, as dogwhelks exposed to air and sunlight have greatly increased body temperatures and the magnitude of these increases are also affected by shell color (Etter, 1988).

Therefore, due to the combination of varying water temperatures, air temperatures, and solar radiation experienced by free-ranging dogwhelks, quantifying true site-specific detection durations under realistic conditions would be challenging. The laboratory conditions probably maximized dogwhelk feeding and minimized dogwhelk digestion rates, so the calculated detection durations may be closer to maximum rather than average durations.

Even after the removal of cross-reacting dogwhelk-mussel precipitin lines, long-term reactions with non-provided prey were also observed (Fig. 4). When averaged across all sample dates, these reactions occurred at relatively low frequencies, though they suggest that immunoassays are more applicable for highlighting differences between sites than for showing differences among individuals within sites. The average 10% difference between prey species (Fig. 4) may be partly due to the >2 precipitin line minimum for a positive test against the anti-mussel antibody being applied to these tests before these proportions were calculated. Eliminating precipitin lines from barnacle reactions to decrease potential false positive reactions, however, is not advantageous. Immunoassays using anti-barnacle antibody yield an average of 1.23 precipitin line immediately following feeding (Table 2; Fig. 2). Therefore, eliminating a single line from any test involving the anti-barnacle antibody would reduce the power to detect predation on barnacles and under such >1 precipitin line limit for positive reactions, 51% of the barnacle-fed dogwhelks that tested positive for barnacles would be considered cross reactions.

Previous analyses of dogwhelk and related species' prey choice in the field have largely relied on either repeated observations of free-ranging animals (Connell, 1961; Fairweather, 1985; Hughes and Drewett, 1985; West, 1986; Hughes and Burrows, 1993) or assessment of prey mortality in cages (Connell, 1961; Murdoch, 1969; Wieters and Navarrete, 1998; Sanford et al., 2003). Obtaining results using repeated observations or cages, however, remains time consuming and logistically challenging at large spatial scales. Observations of free-ranging animals are often skewed in favor of those prey species that require longer handling times before they are ingested (Fairweather and Underwood, 1983) and replacing prey in experimental cages can cause prey densities to vary (Murdoch, 1969). Single observations may also provide only limited data for dogwhelks, which are known to alternate between periods of feeding and resting (Bayne and Scullard, 1978; Hughes and Burrows, 1993). Connell (1961: Table 19), for example, reported that on average only 60% of dogwhelks were observed feeding during three summer sample periods. The current field observations are similar to Connell's (1961) observations, as 50% of dogwhelks observed and collected were not feeding (Fig. 5). The use of polyclonal antibodies overcomes some of these problems and provides an integrated signal of past predation and qualitative data specific enough to distinguish dogwhelk predation on mussels, barnacles or both species. Confirmation of similar detection intervals (Table 2; Fig. 3) validates the application of immunoassays to highlight site-specific patterns of dogwhelk predation.

Based on the Swans Island field samples, some similarities exist between observations and immunoassays, while differences among sites are apparent (Fig. 5). For example, within the J4 site, many sampled quadrats were obviously dominated by small mussels (≤2 cm) and lacked barnacles, while almost all other quadrats and sites contained at least some barnacles and most contained a mixture of these prey. The dominance of mussel feeders only at J4 suggests the potential importance of alternative prey (barnacle) abundance determining site-specific patterns of predation, which has been previously suggested for dogwhelks (Petraitis, 1998) and documented in similar predator-prey assemblages (e.g., Fairweather, 1985; Wieters and Navarrete, 1998; Berlow, 1999). This pattern of predation on mussels was detectable via both observations and immunoassays.

Though observations and immunoassays from field collections demonstrate some similarities, one main advantage of immunoassays over single observations is that by retaining a long term signature of past predation, immunoassays reduce by more than half the proportions whose prey could not be classified in comparison to field observations (Fig. 5). While the overall percentage of dogwhelks positive for mussels via immunoassays was slightly higher (by 6%) than the percentage observed feeding on mussels, for barnacle feeders this difference was 26% higher via immunoassays than observations. These differences between methods and prey species may be due to different prey handling times prior to ingestion, as differences in handling times can skew the observed or 'apparent' diets of predators to include more of the species with longer handling times (Fairweather and Underwood, 1983). As reviewed above, dogwhelks generally require longer handling times for mussels than for barnacles. While observed mussel predation was similar to immunoassay results, barnacle feeders were underestimated by field observations, which is consistent with predation on barnacles being overlooked due to their short handling times (Fairweather and Underwood, 1983).

Even if the percentage of barnacle feeders is discounted by 10% to account for the potential overestimation due to long term prey detection (Fig. 4), this difference between prey types remains. These differences between observations and gut contents reiterate the utility of predation assessments that incorporate more information than provided by single field observations.

A final advantage of the immunoassays over single field observations is their ability to detect multiple prey within individual predators. On Swans Island, both prey were detected in dogwhelks at four sites, though this immunoassay category occurred least frequently at all of these sites. The absence of many multiple prey feeders, however, is consistent with experimental evaluations of dogwhelk foraging behavior. Dogwhelks are slow to switch between mussels and barnacles and consequently dogwhelks are much more efficient at consuming previously encountered prey species (Hughes and Dunkin, 1984; Dunkin and Hughes, 1984; Rovero et al., 1999). Given this aspect of dogwhelk behavior and the demonstrated sensitivity of immunoassays to detect prey for long durations, the absence of multiple prey in dogwhelks is expected and appears to support the application of this method to field collected data.

Given that only one mussel and one barnacle species was tested, it is possible that these field collected dogwhelks might have consumed other species that cross react with these antisera. Feller et al. (1979) described an algorithm for conservatively determining trophic links when many predators and many prey are tested that eliminated potential cross-reactions between prey in predator guts as well as prey with the predator. Such a comparison involving all potential prey species was not performed on dogwhelks, as the cross reacting antigens shared between dogwhelks and mussels were characterized and an abundance of natural history information indicates that dogwhelks feed primarily on these two dominant, sessile prey species (Hughes and Dunkin, 1984, Crothers, 1985; Fisher 2005). It is possible, however, that some of the mussels consumed by dogwhelks or even those used to create the mussel antibody were actually *Mytilus trossulus*, as this species now occurs in the Gulf of Maine intertidal and is morphologically indistinguishable from *M. edulis* (Rawson et al., 2007). Other *Mytilus* species (*M. edulis*, *M. galloprovincialis*, and *M. californianus*) have been shown to react extensively in immunoassays due to many shared antigens (Brock, 1985). Therefore, if dogwhelks consume *M. trossulus*, the immunoassay results may be indistinguishable from *M. edulis* reactions, though the current results did not test this hypothesis.

Having demonstrated the specificities, equal detection durations, and characterized the extent of cross reactions, these polyclonal antibodies not only provide information about dogwhelk behavior at intermediate spatial scales but will allow further evaluations of predation from local to regional spatial scales. Together with samples of local prey abundance, for example, these methods will facilitate formal examinations of the extent and implications of site-specific patterns of predation by dogwhelks at a total of 19 intertidal sites around the Gulf of Maine. Similar patterns of predation by gastropods on few dominant mussel and barnacle species also occur in other intertidal assemblages (e.g., Murdoch, 1969; Hughes and Burrows, 1993; Wieters and Navarrete, 1998; Berlow, 1999; Navarrete and Castilla, 2003). Therefore, in addition to future investigations within the Gulf of Maine, polyclonal antibodies or other molecular methods might similarly be applied to expand the spatial scale of intertidal predation studies at other locations.

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