

RESEARCH NOTES

Individual selection and the evolution of chemical defence in nudibranchs: experiments with whole *Cadlina luteomarginata* (Nudibranchia: Doridina)

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Nudibranchs lack the physical protection of a shell but often possess chemical defence and aposematic coloration.^{1–3} Three types of selection are commonly cited to explain the evolution of warning coloration and chemical defence: kin selection, green-beard selection and individual selection.⁴ Kin selection seems unlikely, as nudibranchs are typically uncommon, dispersed and have pelagic larvae.^{2,5} Models of green-beard selection suggest that uncommon, conspicuous prey are unlikely to survive except in the extreme case of one-trial learning with no forgetting, as might happen with very toxic species.⁴ Although some nudibranchs undoubtedly have extremely toxic secondary chemicals,⁶ their toxicity in most species is unknown.⁷

Alternatively, chemical defence and aposematism may arise by individual selection if the defended individual both repels and survives predator attacks.^{5,8} There is some evidence that slugs are not greatly damaged from attacks by fish,^{8,9} but selection in benthic marine habitats is usually from a diverse suite of omnivorous predators.¹⁰ These may differ not only in their reaction to particular defence types,¹⁰ but also in their attack mode and consequently the amount of damage inflicted on soft-bodied prey during sampling.¹¹ It is therefore worth investigating whether nudibranchs deter and survive attacks from a range of predators besides fish.

I assayed *Cadlina luteomarginata* MacFarland, 1966, a common Northeastern Pacific species with well-characterized secondary chemistry,⁷ against several of the generalist predators I most commonly saw in its habitat. These species either cut and crush, or mouth and swallow their prey when attacking (Table 1). Predators were collected from inlets near Bamfield, British Columbia and transferred to individual aquaria at the Bamfield Marine Station. All were acclimated to laboratory conditions, after which they were fed 2-cm² pieces of squid mantle every other day for *c.* 1 week to train them to receive experimental food. This produced predators that were hungry, but not starving, for bioassays. Nudibranchs were collected from Bamfield by hand, using snorkelling or scuba and were maintained in the laboratory for several days without food to clear their guts of sponge remnants that might have reduced their palatability.

For assays, each predator was presented with food twice: once with squid mantle (the control) and once with one nudibranch. Approximately half of the predators received the control first,

the other half received the nudibranch first. Predators that did not sample the control food (i.e. manipulating it with chelae or taking it into their mouths) were excluded from analysis as being insufficiently motivated to feed. Nudibranchs were recorded as consumed if they were swallowed and not ejected whole during a period of observation appropriate to each predator's mode of feeding: 5 min for crabs and fish, 24 h for anemones and sea stars. Data were analysed using Cochran's Q test.¹² After sampling by predators, nudibranchs were kept in 1-l mesh-sided cages in natural flowing seawater for 1 week, during which survival and slug condition were recorded daily. Several nudibranchs escaped their cages during the observation period, and so were excluded from further consideration.

Only one *C. luteomarginata* was consumed (Table 1), suggesting this species is effectively defended against a range of predators with different feeding modes. Predators rejected whole *C. luteomarginata* much more consistently than in previous experiments that used food treated only with chemical extracts at natural concentrations¹³ or single compounds.^{14,15} This could be because predator responses to particular compounds vary^{10,16,17} or we may not have found the full range of deterrent compounds for this nudibranch. Nudibranchs also have other defences against predators, such as lowered nutritional quality¹⁸ and possibly spicules,^{3,13} so assays of secondary chemicals alone may underestimate the effectiveness of this slug's defence.

Overall survival after attacks was high, but cutting and crushing attacks caused less mortality than mouthing and swallowing (Table 1). Although crabs usually clipped and pierced the mantle with their chelae, no nudibranchs died after sampling and the slugs' wounds began healing within the week. Survival was lowest for slugs attacked by anemones; some were moribund, suggesting damage from nematocysts, and nudibranchs regurgitated during the experiment were often partially digested and did not survive long. Survival after attack by *P. helianthoides* was higher, but depended on whether the nudibranchs were briefly sampled with the tube feet or were actually swallowed (B.K. Penney, unpublished). Survival after fish attacks could not be evaluated due to low sample size; however, in other experiments with fish, slugs have been little damaged.^{8,9} The generally high survival after sampling by predators supports the hypothesis of individual selection for chemical defence. The level of

Table 1. Consumption and survival of *Cadlina luteomarginata* to 7 days post-attack.

Predator	Class	Collection method	Attack type	Eaten	Survival
<i>Cancer productus</i> Randall, 1839	Malacostraca	Baited trap	Cut/crush	*0% (10)	100% (10)
<i>Cancer gracilis</i> Dana, 1852	Malacostraca	Baited trap	Cut/crush	*0% (11)	100% (9)
<i>Hexagrammos decagrammus</i> (Pallas, 1810)	Osteichthyes	Line and hook	Mouth/swallow	*0% (8)	50% (2)
<i>Pycnopodia helianthoides</i> (Brandt, 1835)	Asteroidea	SCUBA	Mouth/swallow	*10% (10)	89% (9)
<i>Anthopleura elegantissima</i> (Brandt, 1835)	Anthozoa	Hand	Mouth/swallow	*0% (9)	67% (9)

For both consumption and survival, data are given as a percentage, with sample sizes in parentheses. *N* for survival only includes nudibranchs monitored to the end of the experiment. *Difference from consumption of control food ($P < 0.05$, Cochran's Q test).

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mortality in cases where slugs are swallowed whole may be ameliorated in nature if these predators are less important than crabs and fish, or if the nudibranchs are less preferred than other available prey.

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Hatchlings of the deep-sea octopus *Graneledone boreopacifica* are the largest and most advanced known

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Although parts of at least four deep-sea octopus egg clutches have been collected in the last 120 years,^{1–3} free-living hatchlings have remained unknown. The remotely operated vehicle (ROV) *Tiburon* documented the hatching of an egg clutch brooded by a female of *Graneledone boreopacifica* Nesis, 1982 at 1,600 m depth on the Gorda Escarpment and collected 28 hatchlings. The 55-mm long hatchlings are the largest and most developmentally advanced octopus hatchlings known, although their external yolk sacs indicate that hatching was premature. The hatchlings carry up to 57 suckers per arm and the hectocotylus of male hatchlings carries the full adult complement of 42 or 43 suckers. The internal yolk sac displaces the small digestive gland to the ventral, posterior mantle cavity, as is typical of embryos of *Octopus vulgaris*.

All observations and collections reported were made with the ROV *Tiburon* on 22 July 2002 as it crossed the north-facing 1570–1600 m deep slope of the Gorda Escarpment.⁴ The octopuses observed were identified as *Graneledone boreopacifica* based on their single, or zigzagged, suckers, clear supra-ocular papillae and the skin texture which corresponded to the faint papillae characteristic of North Pacific specimens of this genus from shallow in their depth range.⁵

A female adjacent to a rock outcrop in the brooding position³ was collected and her exposed egg clutch briefly videotaped. As suction sampling began, mass hatching ensued (Fig. 1). After the ROV was recovered, a brief series of standard measurements was made on the moribund hatchlings (Fig. 2). The specimens,

including the brooding female, an unhatched egg and egg cases were preserved for deposition at The Field Museum (Chicago, IL; FMNH 302123; FMNH 302124; FMNH 302125; FMNH 302302).

In the laboratory, the measurements were replicated after 9 months in alcohol storage. All arm suckers on one side of 17 individuals were counted under a dissecting microscope. The



Figure 1. A hatchling of *Graneledone boreopacifica* swims away as the suction sampler of the ROV *Tiburon* pulls at the egg clutch. Note the external yolk sac within the arm crown of the hatchling.

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