

Skin toxins and external parasitism of coral-dwelling gobies

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Toxic (*Gobiodon* spp.) and non-toxic (*Paragobiodon xanthosomus*) gobies became infected with external parasites (gnathiid isopods) at equal rates in a laboratory experiment. Parasites were evenly distributed over the body of *P. xanthosomus* but were mostly confined to the fins of *Gobiodon* spp., where toxin glands are less abundant. Skin toxins were not associated with the rate of infection but their distribution did appear to influence the site of parasite attachment.

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Many animals possess toxic compounds that can deter predators (Duffy & Hay, 2001), influence competitors (Paul, 1992) or reduce infections of bacteria or parasites (Mouritsen & Madsen, 1994; Koh, 1997; Williams *et al.*, 1998). Toxins are very common among teleosts, either in the form of venom or crinotoxins (Halstead, 1978). Venom is injected into other organisms and is thought to be a predator defence in many marine fishes (Cameron & Endean, 1973; Halstead, 1978; Rifkin & Williamson, 1996). In contrast, crinotoxins are not associated with any venom apparatus and their role in marine fishes is less clear.

Over 50 species of teleosts from 14 families possess crinotoxins (Lassig, 1981) and in many cases these toxins are secreted from the epidermis (Cameron & Endean, 1973; Halstead, 1978). Observations that crinotoxins can kill other fishes in the laboratory (Thomson, 1964; Hashimoto *et al.*, 1974; Kalamanzon & Zlotkin, 2000) and that some toxic species are avoided by predators (Tachibana *et al.*, 1984; Gladstone, 1987) have led to the common assumption that epidermal toxins are widely used as a defence against predation. Toxic skin secretions, however, may have ecological roles other than, or in addition to,

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detering predators. Cameron & Endean (1973) noted that many crinotoxic fishes had a sedentary mode of life and proposed that epidermal toxins in these species may provide protection against non-predatory threats such as parasitism, just as toxins in some other benthic marine taxa deter fouling and parasitic organisms (Wahl, 1989; Pawlik, 1992; Kubanek *et al.*, 2002).

Coral gobies of the genus *Gobiodon* are small (<60 mm total length, L_T), coral-dwelling fishes that produce toxic skin secretions. Although these secretions can be lethal to other fishes (Hashimoto *et al.*, 1974), individuals of most species of *Gobiodon* rarely leave the shelter of their host corals (Munday *et al.*, 2001), where they are likely to be well protected from predation by large fishes. Their sedentary life style may encourage colonization by fouling organisms and parasites. Therefore, in addition to any activity as a predator deterrent (Schubert *et al.*, in press), the skin secretions of these fishes may play a role in defence against parasites or fouling organisms. Unlike *Gobiodon* spp., coral gobies of the genus *Paragobiodon* do not possess skin toxins (Hashimoto *et al.*, 1974; Lassig, 1981). *Paragobiodon* and *Gobiodon* are largely ecologically equivalent, except that *Gobiodon* spp. mostly inhabit corals from the family Acroporidae (Munday *et al.*, 1997), whereas *Paragobiodon* spp. mostly inhabit corals from the family Pocilloporidae (Tyler, 1971). The presence of toxic and non-toxic sister taxa, with similar ecological characteristics, provided an opportunity to test the possible role of skin toxins in mediating parasite–host interactions in small, sedentary reef fishes.

Species of *Gobiodon* and *Paragobiodon* are similar in body size and shape but differ in the structure of their integument. *Gobiodon* have no scalation but possess epidermal toxin glands that surround the body, except for the fins (Fig. 1). In contrast, *Paragobiodon* have scales but lack epidermal toxin glands. Field observations support the hypothesis that the presence of skin toxins influences the vulnerability to parasitism of coral-dwelling gobies. No external parasites were found on *Gobiodon histrio* (Valenciennes) ($n=37$), *Gobiodon okinawae* Sawada ($n=4$) or *Gobiodon unicolor* (Castelnau) ($n=22$) collected from Lizard island ($14^{\circ}40'S$; $145^{\circ}28'E$) on the Great Barrier Reef, whereas, 41% of *Paragobiodon xanthosomus* (Bleekers) ($n=17$), from the same location were infected with external parasites (pers. obs.).

In this study, laboratory experiments were conducted to test whether: (1) toxic coral gobies (*Gobiodon* spp.) were less susceptible than non-toxic coral gobies (*Paragobiodon* spp.) to infection by a common external fish-parasite (gnathiid isopods), (2) the site of parasite attachment differed between toxic and non-toxic coral gobies, and (3) differences were associated with the distribution of toxin glands. Gnathiid isopods were used because they are one of the most common external parasites of reef fishes (Grutter & Poulin, 1998) and they are visible to the naked eye when attached to the host, making infection rates and attachment sites easy to determine. Gnathiid isopods were bred in captivity (Grutter, 2001) and had no prior experience of coral gobies. All gnathiids were third developmental stage juveniles. Three toxic species of goby, *G. okinawae*, *G. histrio* and *G. unicolor*, and one non-toxic species, *P. xanthosomus*, were used in the experiments. Gobies were collected from the wild at Lizard Island after anaesthetization with clove oil (Munday & Wilson, 1997).

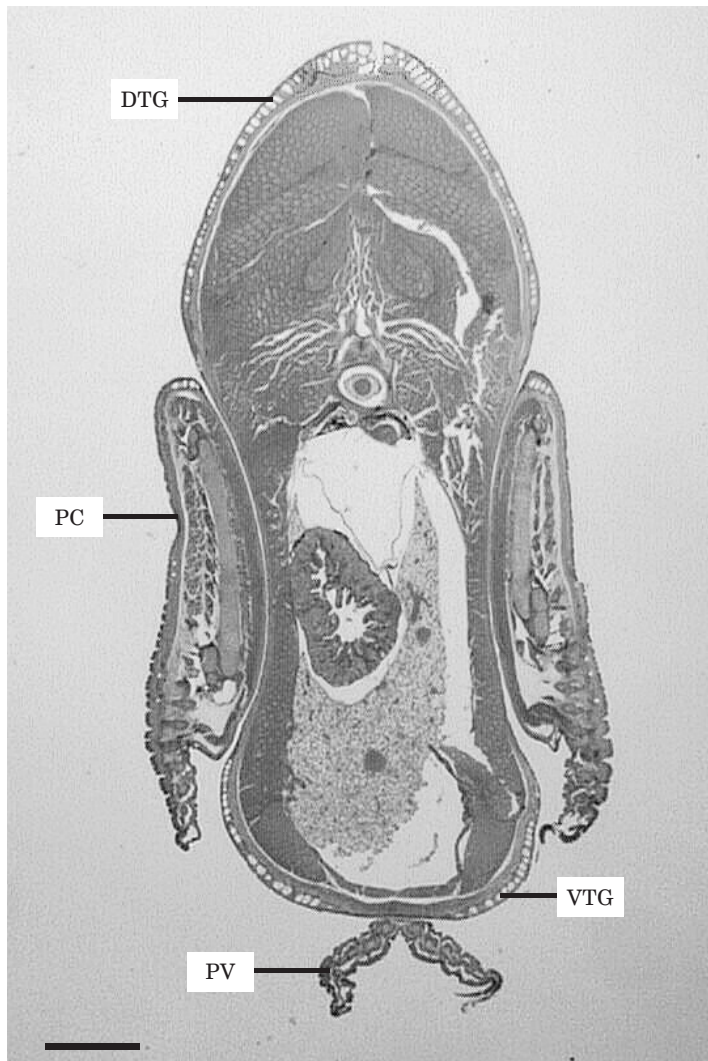


FIG. 1. Transverse section of *Gobiodon histrio* showing the dense distribution of toxin secretory glands on the dorsal (DTG) and ventral (VTG) parts of the body and reduced distribution of glands on the pectoral (PC) and pelvic fin (PV). Scale bar = 1 mm.

To compare infection rates among toxic and non-toxic gobies, gnathiid isopods were given the choice of *Gobiodon* and *Paragobiodon* as potential hosts. One *Gobiodon* spp. and one *P. xanthosomus*, of approximately equal size, were placed simultaneously into a beaker containing 500 ml of sea water and five gnathiid isopods. The gnathiids were observed until all had attached to a fish and the species of fish with the greatest number of gnathiids attached was recorded. Trials were replicated 10 times for each species of *Gobiodon*, and new fishes and gnathiids were used in each trial.

All parasites attached to a fish within 10 min, but there was no statistically significant difference in the infection rate of the three species of *Gobiodon*

compared to *P. xanthosomus*. A greater number of isopods attached to the toxic *Gobiodon* species in 12 trials and a greater number of isopods attached to the non-toxic *P. xanthosomus* in 18 trials ($P=0.27$). Also, the total number of isopods attaching to each species of *Gobiodon* did not differ from the total number of isopods attaching to *P. xanthosomus* (23 on *G. histrio* v. 27 on *P. xanthosomus*, $P=0.57$; 23 on *G. okinawae* v. 27 on *P. xanthosomus*, $P=0.57$; 21 on *G. unicolor* v. 29 on *P. xanthosomus*, $P=0.57$). Therefore, the presence of skin toxins in species of *Gobiodon* did not appear to influence the likelihood of parasite infection compared to the non-toxic *P. xanthosomus*.

Toxin glands were absent from the gills of *Gobiodon* spp. and were less abundant on the fins compared to other parts of the body (Fig. 1). Although the presence of toxins did not influence the overall rate of infection between toxic and non-toxic gobies, the uneven distribution of skin toxins may influence the site of attachment by parasites. Therefore, in the second experiment, the site of attachment by gnathiid isopods on each of the three toxic gobies, *G. okinawae*, *G. histrio* and *G. unicolor*, was compared to that on the non-toxic goby, *P. xanthosomus*. At the start of each trial, one goby was placed into a beaker containing 500 ml of sea water with five gnathiid isopods. The sites of attachment were determined after all isopods had attached to the fish. Attachment sites were classified as fins, body or gills. Ten trials were conducted for each species of *Gobiodon* and for *P. xanthosomus*. To determine if gnathiids preferentially attached to particular regions of the toxic and non-toxic fishes, the surface area of the body and the fins was estimated digitally using *G. histrio* and *P. xanthosomus* as standards. The distribution of gnathiids attached to the fins v. the body was then compared for each species of goby using a χ^2 goodness of fit test, where the expected values were adjusted to the proportion of surface area of fins and the remainder of the body.

All gnathiids had attached to the fish and had begun to engorge with blood within 15 min. Gnathiids mostly attached to the fins of each *Gobiodon* species (Fig. 2). In contrast, they attached more frequently to the body of *P. xanthosomus*

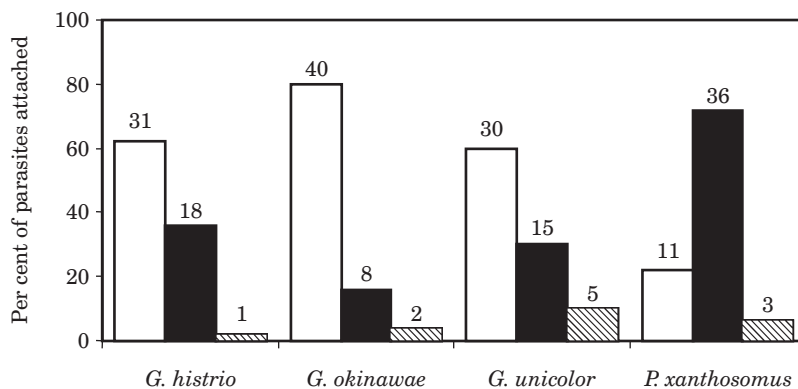


FIG. 2. Per cent of gnathiid isopod parasites that attached to the fins (□), body (■) and gills (▨) of *Gobiodon histrio*, *G. okinawae*, *G. unicolor* and *Paragobiodon xanthosomus*. Total number of isopods attached at each location is shown.

(Fig. 2). Gnathiids attached to the gills of *Gobiodon* species and *P. xanthosomus* in low but approximately equal frequency (Fig. 2). When surface area was considered, gnathiids attached more frequently than expected to the fins compared to the body of all three species of *Gobiodon* (*G. okinawae*, $\chi^2 = 28.2$, d.f. = 1, $P < 0.001$; *G. histrio*, $\chi^2 = 8.7$, d.f. = 1, $P < 0.005$; *G. unicolor*, $\chi^2 = 20.4$, d.f. = 1, $P < 0.001$). In contrast, they attached in proportion to the surface area of fins and body in *P. xanthosomus* ($\chi^2 = 1.2$, $P > 0.3$).

Although skin toxins have been implicated in reducing parasitic loads in some animals (Mouritsen & Madsen, 1994; Williams *et al.*, 1998) this does not appear to be the case for coral gobies. Skin secretions from species of *Gobiodon* did not reduce the rate of infection by a common external fish-parasite, but did appear to influence the site of attachment by the parasites. More parasites attached to the fins where toxin glands were sparsely distributed, compared to the body where toxin glands were densely distributed. The uneven distribution of parasites on the host fish may be biologically relevant because the fins are less vascularized than the body surface. Therefore, parasites that attach to fins, rather than the body, may have less impact on the host fish. The number of parasites attaching to the gills was low for both toxic and non-toxic species, which may reflect the protection offered by the operculum. The operculum of coral gobies is restricted in length (Harold & Winterbottom, 1995) and is sealed by a flap of skin when closed (pers. obs.). The small, well-sealed opercular opening may pose a significant barrier to parasite entry.

Gobiodon skin secretions are highly toxic to other fishes and may be an important predator deterrent (Schubert *et al.*, in press). The correlation between the distribution of toxin glands and the site of parasite attachment demonstrated here indicates that the same toxins appear to influence parasite–host interactions. Therefore, like chemical defences in other organisms (Schmitt *et al.*, 1995; Kubanek *et al.*, 2002), they too may have multiple ecological roles.

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References

- Cameron, A. M. & Endean, R. (1973). Epidermal secretions and the evolution of venom glands in fishes. *Toxicon* **11**, 401–410.
- Duffy, J. E. & Hay, M. E. (2001). The ecology and evolution of marine consumer–prey interactions. In *Marine Community Ecology* (Bertness, M. D., Gaines, S. D. & Hay, M. E., eds), pp. 131–151. Sunderland: Sinauer Associates.
- Gladstone, W. (1987). The eggs and larvae of the sharp-nose pufferfish *Canthigaster valentini* (Pisces: Tetraodontidae) are unpalatable to other reef fishes. *Copeia* **1987**, 227–230.
- Grutter, A. S. (2001). Parasite infection rather than tactile stimulation is the proximate cause of cleaning behaviour in reef fish. *Proceedings of the Royal Society of London Series B* **268**, 1361–1365.
- Grutter, A. S. & Poulin, R. (1998). Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fish. *Marine Ecology Progress Series* **164**, 263–271.
- Halstead, B. W. (1978). *Poisonous and Venomous Marine Animals of the World*. New Jersey: The Darwin Press.

- Harold, A. S. & Winterbottom, R. (1995). *Gobiodon acicularis*, a new species of gobioid fish (Teleostei: Gobiidae) from Belau, Micronesia. *Proceedings of the Biological Society of Washington* **108**, 687–694.
- Hashimoto, Y., Shiomi, K. & Aida, K. (1974). Occurrence of a skin toxin in coral-gobies *Gobiodon* spp. *Toxicon* **12**, 523–528.
- Kalamanzon, E. & Zlotkin, E. (2000). An ichthyotoxic protein in the defensive skin secretion of the Red Sea trunkfish *Ostracion cubicus*. *Marine Biology* **136**, 471–476.
- Koh, E. G. L. (1997). Do scleractinian corals engage in chemical warfare against microbes? *Journal of Chemical Ecology* **23**, 379–398.
- Kubaneck, J., Whalen, K. E., Engel, S., Kelly, S. R., Henkel, T. P., Fenical, W. & Pawlik, J. R. (2002). Multiple defensive roles for triterpene glycosides from two Caribbean sponges. *Oecologia* **131**, 125–136.
- Lassig, B. R. (1981). Significance of the epidermal ichthyotoxic secretion of coral-dwelling gobies. *Toxicon* **19**, 729–735.
- Mouritsen, K. N. & Madsen, J. (1994). Toxic birds: defence of parasites? *Oikos* **69**, 357–358.
- Munday, P. L. & Wilson, S. K. (1997). Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacanthus amboinensis*, a coral reef fish. *Journal of Fish Biology* **51**, 931–938.
- Munday, P. L., Jones, G. P. & Caley, M. J. (1997). Habitat specialisation and the distribution and abundance of coral-dwelling gobies. *Marine Ecology Progress Series* **152**, 227–239.
- Munday, P. L., Jones, G. P. & Caley, M. J. (2001). Interspecific competition and coexistence in a guild of coral-dwelling fishes. *Ecology* **82**, 2177–2189.
- Paul, V. J. (1992). Chemical defences of benthic marine invertebrates. In *Ecological Roles of Marine Natural Products* (Paul, V. J., ed.), pp. 164–188. New York: Cornell University Press.
- Pawlik, J. R. (1992). Chemical ecology of the settlement of marine invertebrates. *Oceanography and Marine Biology, an Annual Review* **30**, 273–335.
- Rifkin, J. & Williamson, J. (1996). Venomous fish. In *Venomous and Poisonous Marine Animals: a Medical and Biological Handbook* (Williamson, J. A., Fenner, P. J., Burnett, J. W. & Rifkin, J. F., eds), pp. 354–395. Sydney: University of New South Wales Press.
- Schmitt, T. M., Hay, M. E. & Lindquist, N. (1995). Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. *Ecology* **76**, 107–123.
- Schubert, M., Munday, P. L., Caley, M. J., Jones, G. P. & Llewellyn, L. E. (in press). The toxicity of skin secretions from coral-dwelling gobies and their potential role as a predator deterrent. *Environmental Biology of Fishes*.
- Tachibana, K., Sakaitani, M. & Nakanishi, K. (1984). Pavoninins: shark-repelling ichthyotoxin from the defence secretion of the Pacific sole. *Science* **226**, 703–705.
- Thomson, D. A. (1964). Ostracitoxin: an ichthyotoxic stress secretion of the boxfish, *Ostracion lentiginosus*. *Science* **146**, 244–245.
- Tyler, J. C. (1971). Habitat preferences of the fishes that dwell in shrub corals on the Great Barrier Reef. *Proceedings of the Academy of Natural Sciences of Philadelphia* **123**, 1–26.
- Wahl, M. (1989). Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series* **58**, 175–189.
- Williams, C. R., Wallman, J. F. & Tyler, M. J. (1998). Toxicity of green tree frog (*Litoria caerulea*) skin secretion to the blowflies *Calliphora stygia* (Fabricius) and *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). *Australian Journal of Entomology* **37**, 85–89.