

# Fish mucous cocoons: the 'mosquito nets' of the sea

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**Mucus performs numerous protective functions in vertebrates, and in fishes may defend them against harmful organisms, although often the evidence is contradictory. The function of the mucous cocoons that many parrotfishes and wrasses sleep in, while long used as a classical example of antipredator behaviour, remains unresolved. Ectoparasitic gnathiid isopods (Gnathiidae), which feed on the blood of fish, are removed by cleaner fish during the day; however, it is unclear how parrotfish and wrasse avoid gnathiid attacks at night. To test the novel hypothesis that mucous cocoons protect against gnathiids, we exposed the coral reef parrotfish *Chlorurus sordidus* (Scaridae) with and without cocoons to gnathiids overnight and measured the energetic content of cocoons. Fish without mucous cocoons were attacked more by gnathiids than fish with cocoons. The energetic content of mucous cocoons was estimated as 2.5 per cent of the fish's daily energy budget fish. Therefore, mucous cocoons protected against attacks by gnathiids, acting like mosquito nets in humans, a function of cocoons and an efficient physiological adaptation for preventing parasite infestation that is not used by any other animal.**

**Keywords:** coral reefs; Gnathiidae; Scaridae; parasites; mucus; behaviour

## 1. INTRODUCTION

The functions of the thin layer of mucus covering fishes include osmoregulation, reducing friction and protection from abrasions, pollutants and desiccation [1], as well as ultraviolet radiation [2]. The role of mucus in protecting fish from harmful organisms, however, is uncertain [1]. One of the most notable nocturnal behaviours of coral reef fishes, mainly some wrasses and parrotfishes, is the large mucous cocoons that they envelop themselves in at night [3–5]. The function of mucous cocoons remains unresolved. In an early study, showing that spotted moray eels (*Gymnothorax moringa*) ate more of three species that do not secrete cocoons (*Sparisoma radians*, *Sparisoma chrysopterum* and *Cyprinodon* sp.) than a parrotfish species that does (*Scarus croicensis*), Winn & Bardach [6] 'tentatively' (p. 298) concluded that cocoons reduce predation by the spotted moray eel. However, the effect of species differences was not controlled for nor was mucous cocoon presence manipulated, and many individuals of the cocoon-producing species were still eaten during the experiment. Indeed, the

role of mucous cocoons in large wrasse as defence against predators while wedged in crevices or buried in sand has been questioned [7,8]. Alternative functions proposed include protection against settling silt [4] and bacteria [9], and as a warning system upon contact [9]. Despite limited empirical support, the function of cocoons as defence against nocturnal predators remains regularly cited [10,11].

Gnathiid isopods attack many coral reef fishes, especially at night [12–14]. During the day, parrotfish repeatedly seek cleaner fish [15], which only control gnathiid infestations during the day [13] but it is not clear how parrotfish control gnathiids at night. Here, we tested the hypothesis that the production of a mucous cocoon in the bullethead parrotfish (*Chlorurus sordidus*) is an energetically efficient and effective means to reduce nocturnal attacks by gnathiids, parasites that harm fish in various ways [16,17].

## 2. MATERIAL AND METHODS

*Chlorurus sordidus* were collected with a barrier net at Lizard Island Great Barrier Reef held singly in 65 × 40 × 36 cm plastic bins, and supplied with a shelter (11 diameter × 13 cm pipe), constant sea water and aeration. Mucous cocoon production began at the mouth and progressed backwards to surround the body in a solid gelatinous mass in 45–60 min. In order to test whether cocoons defend against gnathiids, fish were placed singly in the bins on their first night of captivity (15–19 March 2004) and randomly assigned to a cocoon presence treatment. At midnight, when all fish had produced a cocoon, the cocoon was removed from half of the fish by gently pushing the fish out of its cocoon and scooping out the mucus with a scoop. Fish remained asleep during this procedure. Twenty, cultured [14], unfed third stage gnathiids *Gnathia aureusmaculosa* were then added to each bin. Fish were exposed to gnathiids for 4.5 h, this is sufficient time for the gnathiids to feed off the fish *Hemigymmus melapterus* [14]. Flow-through water was not supplied from 18.00 to 06.00 h to contain gnathiids in bins. Beginning at 04.30 h, quiescent fish were gently rubbed to remove any attached gnathiids and fish were removed with a hand net. Gnathiids in the bin were collected with a sieve (62 µm) and preserved in 10 per cent formalin. Fed gnathiids were identified by their engorged red gut [14]. Gnathiid feeding success was expressed as the number of fed gnathiids relative to the total number recovered (14–20). Trials in which the fish had abandoned or damaged its cocoon ( $n = 5$ ) or produced a second cocoon after the first one was removed ( $n = 7$ ) were omitted from the analyses.

The mucous cocoons removed above were dried to a constant mass at 70 °C in an oven. Organic content was determined by then burning the cocoon in a muffle furnace (500 °C) for 24 h, and subtracting the mass after combustion from the pre-combustion dry mass. Gross energy (GE) values of mucous cocoons, obtained from fish collected in September 2009, were obtained using a bomb calorimeter (IKA—WERKE, C2000, GMSBH & CO. KG, Staufen, Germany), which had been standardized using benzoic acid. Since individual cocoons contained extremely low energy and would not combust alone, they were mixed with a known amount of sunflower oil (0.4 g) of a known energy content (mean ± s.e., 39 586 ± 1 J g<sup>-1</sup>,  $n = 3$ ). GE was measured individually for three similar-sized fish (146 ± 16.3 g). The mean of two to four replicates per sample was used.

## 3. RESULTS

Significantly, more fish from which the cocoon had been removed (94.4%), compared with fish with cocoons not removed (10%), were attacked by gnathiids (likelihood ratio test,  $\chi^2 = 31.9$ ,  $p < 0.001$ ). The median proportion of gnathiids that had fed on fish was higher on fish without than fish with cocoons (Wilcoxon rank sum test,  $S = 519.5$ ,  $Z = 5.25$ ,  $p < 0.0001$ ; figure 1). Many fish (60%) produced a second cocoon after the first one was removed at midnight. The mass per cocoon, estimated using organic

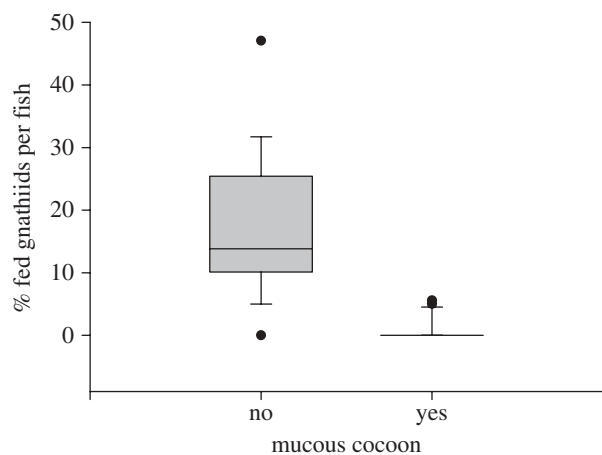


Figure 1. The percentage of fed gnathiids in trials with ( $n = 20$ ) and without mucous cocoons ( $n = 18$ ). Box and whisker plot: centre line denotes the median value, box encloses the inner two quartiles, error bars indicate the 90th and 10th percentiles and the closed circles indicate outliers.

content, increased with fish body mass with a mass exponent of 0.29 (cocoon mass (mg) =  $0.107 Mb^{0.29}$ , where  $Mb$  = body mass (g)), thus indicating a relatively higher cost of cocoon production for smaller fish. The energetic content (mean  $\pm$  s.e.) of cocoons with a mass of  $146 \pm 16.3$  g was  $861 \pm 42$  J per cocoon; when adjusted to the mass of dried cocoon (which included much salt) it was  $469 \pm 123$  J g<sup>-1</sup>. Although the daily energy budget is not available for *C. sordidus*, the daily energy assimilation is for the parrotfish *Scarus vetula* [18]; this measure, however, does not include costs like basal metabolic rate, foraging and digesting food. Using this measure ( $34\,000$  J d<sup>-1</sup>) for *S. vetula* with a mass of 134 g as a proxy for the daily energy budget, and the average energetic content per cocoon of the above *C. sordidus*, we estimated that it cost *C. sordidus* 2.5 per cent of their daily energy budget to produce a cocoon

#### 4. DISCUSSION

By remaining in a mucous cocoon at night, parrotfish *C. sordidus* may avoid being attacked by gnathiids, which regularly attack fish at night [13] and possibly other parasitic isopods, of which there is a wide range on coral reefs [19]. This anti-ectoparasitic protective function differs from Winn & Bardach's [6] tentative proposal that cocoons defend against predatory moray eels, but the two functions need not be mutually exclusive. Cocoons may prevent infestation by masking olfactory cues used by gnathiids to find fish or act as a physical or chemical barrier. Gnathiids can be harmful when in large numbers [16], and have been implicated as vectors of haemogregarines, blood parasites found in a few coral reef fishes, including parrotfishes [17].

How fishes that do not produce mucous cocoons prevent nocturnal isopod attacks is unclear; however, some gobies have toxic skin cells that prevent gnathiid attacks on certain body areas [20], many wrasses and some parrotfish bury themselves in the sand [5,21], a

parrotfish (*Sparisoma aurofrenatum*) that does not produce cocoons sleeps in open areas away from the reef [22]; alternatively, fish may use nocturnal cleaner organisms. While noxious compounds occur in fish mucus [1], whether they do in cocoons is unclear.

The larger allocation of organic content to cocoons by smaller fish suggests it costs these more to produce cocoons and also that cocoons may have a minimum thickness around the fish. The moderate investment in cocoon production, estimated at 2.5 per cent of their daily energy budget, by using the daily assimilation rate of *S. vetula* [18] as a proxy may explain why fish can produce cocoons nightly and also could produce a second cocoon on the same night if needed. Despite the differences in feeding behaviour between members of these genera [23], the assimilation rate estimated by Bruggemann is a useful starting point for implying daily costs in similar-sized species.

Anti-parasite behaviours fish can engage in, including seeking cleaner organisms, avoiding infectious habitats and infected individuals [24], chafing along a substrate, reducing activity and shoaling [25] are relatively energetically costly. Using mucous cocoons, however, circumvents this limitation by deterring parasites in a moderately energetically efficient way. While aestivating frogs, salamanders and lungfish produce cocoons, this is to prevent desiccation [26].

In contrast to the astonishingly diverse behavioural adaptations [24] and the use of toxic compounds in other animals [27], parrotfish use a physiological adaptation to deter parasites. This involves large highly specialized glands in the gill cavity and/or under the operculum [3], which secrete a structure that not only protects the whole fish but also allows the fish to sleep, a combination of features not known to occur in any other animal. Physiological adaptations to control ectoparasites and other fouling organisms in animals are relatively rare and tend to involve chemical compounds [27]. Mucous cocoons, in contrast, are more reminiscent of the barriers, such as mosquito nets, constructed by humans to control biting arthropods [28]. This adaptation shows the tremendous selective pressure that parasites can impose on fish.

Many thanks to G. Muñoz, C. Jones, J. Pickering, L. Curtis, J. Marshall, N. Raihani, J. Oates, R. Bshary, C. Newport and Lizard Island Research Station staff for their help in the field or on the manuscript, H. Bruggeman for discussions on this project, X. Li for the bomb calorimetry and the Australian Research Council for funding (A.S.G.).

- 1 Shephard, K. L. 1994 Functions for fish mucus. *Rev. Fish Biol. Fish.* **4**, 401–429. (doi:10.1007/BF00042888)
- 2 Zamzow, J. & Losey Jr, G. S. 2002 Ultraviolet radiation absorbance by coral reef fish mucus: photo-protection and visual communication. *Environ. Biol. Fish.* **63**, 41–47. (doi:10.1023/A:1013846816869)
- 3 Casimir, M. J. 1971 Zur Morphologie, Histochemie, Tagesperiodik und Biologie der Operculardrüse bei Labriden und Scariden (Pisces). *Mar. Biol.* **8**, 126–146. (doi:10.1007/BF00350928)
- 4 Winn, H. E. 1955 Formation of a mucous envelope at night by parrot fishes. *Zoologica* **40**, 145–148.

- 5 Randall, J. E., Allen, G. R. & Steene, R. C. 1997 *Fishes of the Great Barrier Reef and Coral Sea*, 2nd edn. Bathurst, Australia: Crawford House Publishing.
- 6 Winn, H. E. & Bardach, J. E. 1959 Differential food selection by moray eels and a possible role of the mucous envelope of parrot fishes in reduction of predation. *Ecology* **40**, 296–298. (doi:10.2307/1930041)
- 7 Potts, G. W. 1973 The ethology of *Labroides dimidiatus* (Cuv. & Val.) (Labridae, Pisces) on alibaba. *Anim. Behav.* **21**, 250–291. (doi:10.1016/S0003-3472(73)80068-5)
- 8 Wiley, J. W. 1974 Observations on the use of mucus envelopes by the California sheephead, *Pimelometopon pulchrum*, on Southern California rock reefs. *Copeia* **1974**, 789–790. (doi:10.2307/1442700)
- 9 Videler, H., Geertjes, G. J. & Videler, J. J. 1999 Biochemical characteristics and antibiotic properties of the mucous envelope of the queen parrotfish. *J. Fish Biol.* **54**, 1124–1127. (doi:10.1111/j.1095-8649.1999.tb00864.x)
- 10 Langerhans, R. B. 2007 Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. In *Predation in organisms*, pp. 177–220. Berlin, Germany: Springer.
- 11 Smith, R. J. F. 1997 Avoiding and deterring predators. In *Behavioural ecology of fishes* (ed. J. G. Godin), pp. 163–190. Oxford, UK: Oxford University Press.
- 12 Grutter, A. S. & Poulin, R. 1998 Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. *Mar. Ecol. Prog. Ser.* **164**, 263–271. (doi:10.3354/meps164263)
- 13 Grutter, A. S. 1999 Cleaner fish really do clean. *Nature* **398**, 672–673. (doi:10.1038/19443)
- 14 Grutter, A. S. 2003 Feeding ecology of the fish ectoparasite, *Gnathia* sp. (Crustacea: Isopoda), from the Great Barrier Reef, Australia and its implications for fish cleaning behaviour. *Mar. Ecol. Prog. Ser.* **259**, 295–302. (doi:10.3354/meps259295)
- 15 Grutter, A. S. 1995 The relationship between cleaning rates and ectoparasite loads in coral reef fishes. *Mar. Ecol. Prog. Ser.* **118**, 51–58. (doi:10.3354/meps118051)
- 16 Jones, C. M. & Grutter, A. S. 2005 Parasitic isopods (*Gnathia* sp.) reduce haemocrit in captive blackeye thicklip (Labridae) on the Great Barrier Reef. *J. Fish Biol.* **66**, 860–864. (doi:10.1111/j.0022-1112.2005.00640.x)
- 17 Smit, N. J., Grutter, A. S., Adlard, R. D. & Davies, A. J. 2006 Hematozoa of teleosts from Lizard Island, Australia, with some comments on their possible mode of transmission and the description of a new hemogregarine species. *J. Parasitol.* **92**, 778–788. (doi:10.1645/GE-756R.1)
- 18 Bruggemann, J. H. 1994 *Parrotfish grazing on coral reefs*. Wageningen, The Netherlands: Posen & Looijen bv.
- 19 Jones, C. M., Miller, T. L., Grutter, A. S. & Cribb, T. H. 2008 Natatory-stage cymothoid isopods: description, molecular identification and evolution of attachment. *Int. J. Parasitol.* **38**, 477–491. (doi:10.1016/j.ijpara.2007.07.013)
- 20 Munday, P. L., Schubert, M., Jones, G. P., Caley, M. J. & Grutter, A. S. 2003 Skin toxins and external parasitism of coral-dwelling gobies. *J. Fish Biol.* **62**, 976–981. (doi:10.1046/j.1095-8649.2003.00078.x)
- 21 DeLoach, N. 1999 *Reef fish behavior: Florida, Caribbean, Bahamas*. Jacksonville, FL: New World Publications, Inc.
- 22 Dubin, R. E. & Baker, J. D. 1982 Two types of cover-seeking behavior at sunset by the princess parrotfish, *Scarus taeniopterus*, at Barbados, West-Indies. *Bull. Mar. Sci.* **32**, 572–583.
- 23 Bellwood, D. R. 1994 A phylogenetic study of the parrotfishes family Scaridae (Pisces: Labroidei), with a revision of genera. *Rec. Aust. Mus. Suppl.* **20**, 1–86. (doi:10.3853/j.0812-7387.20.1994.51)
- 24 Loehle, C. 1995 Social barriers to pathogen transmission in wild animal populations. *Ecology* **76**, 326–335. (doi:10.2307/1941192)
- 25 Wisenden, B. D., Goater, C. P. & James, C. T. 2009 Behavioural defenses against parasites and pathogens. In *Fishes defenses* (eds G. Zaccane, M. A. Perrière & B. G. Kapoor), pp. 151–168. Enfield, NH: Science Publishers.
- 26 Secor, S. M. & Lignot, J. H. 2010 Morphological plasticity of vertebrate aestivation. In *Aestivation: molecular and physiological aspects* (eds C. A. Navas & J. E. Carvalho), pp. 183–208. Berlin, Germany: Springer.
- 27 Williams, C. R., Smith, B. P. C., Best, S. M. & Tyler, M. J. 2006 Mosquito repellents in frog skin. *Biol. Lett.* **2**, 242–245. (doi:10.1098/rsbl.2006.0448)
- 28 Kitchen, L. W., Lawrence, K. L. & Coleman, R. E. 2009 The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. *J. Vector Ecol.* **34**, 50–61.