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**The evolution of mutualism between alpheid shrimp and gobiid fishes: a balance between
benefits and costs**

A Dissertation Presented

by

Patrick J. Lyons

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Abstract of the Dissertation

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I describe several assays designed to examine how costs and benefits interact in the development of mutualisms between species. A mutualism occurs between alpheid shrimp and gobiid fishes. These shrimp have poor vision but good burrowing ability. Individual shrimp share their burrows with a goby that, with good vision but no burrowing ability, acts as a watch-out warning shrimp when predators approach. In the Caribbean, a single species, *Nes longus*, which has been described as a mutualist, follows these behaviors. Others, such as *Ctenogobius saepepallens*, casually use shrimp burrows, rarely warn shrimp of danger, and are better described as commensalists. I found that *N. longus* more effectively avoids predators while using shrimp burrows than *C. saepepallens*. Thus, tight mutualism with shrimp is advantageous, especially in areas where shrimp burrows are abundant. I have quantified several behaviors that likely allow *N. longus* to use burrows more effectively. Why then would *C. saepepallens* not evolve such behaviors and become a strict mutualist if strict mutualism is advantageous? For gobies, there is

likely a cost associated with mutualism with shrimp. To warn shrimp, gobies must remain at a burrow entrances and restrict foraging to that small area. I found that on the same restricted diet, *C. saepepallens* lost more weight than *N. longus*. Thus, *C. saepepallens* may be constrained to a casual association with shrimp due to foraging requirements. This story indicates that strict mutualism may evolve infrequently because few species can overcome the inherent costs of mutualism.

Frontispiece



Table of Contents

List of Tables	vii
List of Figures.....	viii
Chapter 1: Differences in mutualism dependency and their relation to the understanding of paths to obligate mutualism	1
1.1 Introduction.....	2
1.2 Methods.....	7
1.3 Results.....	12
1.4 Discussion	15
1.5 Literature Cited	20
Chapter 2. The benefit of mutualism to obligate and facultative mutualists in a shrimp-goby association.....	31
2.1 Introduction.....	32
2.2 Methods.....	34
2.3 Results.....	39
2.4 Discussion.....	40
2.5 Literature Cited	44
Chapter 3. Foraging requirements as a constraint on a shrimp-goby mutualism.....	58
3.1 Introduction.....	59
3.2 Methods.....	62
3.3 Results.....	68
3.4 Discussion.....	72
3.5 Literature Cited	76
4. Bibliography	85

List of Tables

<u>Table</u>	<u>Page</u>
Table 1.1 Two-way permutation MANOVA with the dependent variable time spent in each the 11 different positions of the reference grid.	27
Table 1.2 Results of a two-way ANOVA with species and location as independent variables and shrimp emergence times from a burrow with the focal goby in position A as the dependent variable.	28
Table 2.1 Results of multiple one-way ANOVAs on different variables related to goby reaction to a model predator.	50
Table 2.2 Results from <i>in situ</i> observations of gobies on two variables: re-emergence time (duration spent in a burrow after being frightened) and flight initiation distance (distance from a SCUBA diver when a focal goby began its retreat).	52
Table 2.3 Results of two-way permutation MANOVA with species and site as independent variables.	53
Table 3.1 Results of a three-way permutation ANOVA with total invertebrate abundance in digestive tract contents of gobies as the dependent variable.	80
Table 3.2 ANCOVA with <i>percent mass loss</i> as the dependent variable.	81

List of Figures

<u>Figure</u>	<u>Page</u>
Figure 1.1 Positions used to quantify goby location and burrow use.	29
Figure 1.2 Mean time (\pm 95% CI) gobies spent in each position.	30
Figure 2.1 Orientation of a focal goby in relation to the model predator that moves from right to left.	54
Figure 2.2 Mean survival time for the two goby species using three different shelter types during staged interactions with a live predator in 190-l aquaria.	55
Figure 2.3 Left axis: flight initiation distance for the two goby species and two sites. Right axis: emergence time (after being frightened into a burrow) for the two goby species.	56
Figure 2.4 Average time spent at different distances-to-shelter.	57
Figure 3.1 Mean abundance of sediment infauna from each of four invertebrate taxa as well as total abundance of all taxa combined.	82
Figure 3.2 Total gut contents of both goby species at both sites.	83
Figure 3.3 Comparisons of digestive tract content of gobies with sediment infauna from shrimp burrow entrances using barplots and Principal Components Analysis (PCA).	84

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Chapter 1: Differences in mutualism dependency and their relation to the understanding of paths to obligate mutualism

Abstract Alpheid shrimps and gobiid fishes engage in a mutualism in which a shrimp, which has poor vision, constructs a burrow that it shares with a goby, which has good vision. The goby in turn, provides the shrimp with information on predators outside the burrow. I compared the behavior of three goby species toward a single shrimp species. The associations between the shrimp and three gobies have previously been described as commensalism, facultative mutualism, and obligate mutualism. I found that the obligate goby spent the most time communicating with shrimp, had greatest preference for the shelter provided by shrimp, and spent the longest durations with individual shrimp before switching to another host. The facultative mutualist was intermediate between the obligate and commensal species for each of these behaviors save the last, in which it was similar to the commensal species. From these findings, I conclude that increasing reliance on a mutualist partner species is matched with increasing preference for the resource the mutualist partner provides over other resources and increasing preference for the resource provided by a mutualist partner necessitates provisioning of better quality and quantity of resources to the mutualist partner. An inability of a species to evolve means of provisioning better resources may inhibit species from evolving more dependent associations with their mutualist partners.

1.1 Introduction

Mutualism is an interspecific interaction benefiting both associating species (Boucher et al. 1982). A long-standing quandary is how these associations could arise given the constraint that natural selection should only favor only traits beneficial to the organisms harboring them and not other individuals (Williams 1996). Darwin wrote, “*If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection*” (Darwin 1859). Thus, an important focus of the study of evolutionary change should be clarification of the conditions that allow mutualism to evolve.

A potentially useful framework for studying the evolution of mutualism is a comparison of obligate and facultative mutualist and commensal species (Roughgarden 1975). An *obligate* mutualist species cannot persist without the resource provided by its partner species (Wolin 1985). A facultative mutualist derives a benefit from resources that its partner species provides, but does not depend solely upon those resources (Wolin 1985). Facultative mutualists maintain the ability to gain the resource through alternative means, whereas obligate mutualists either cannot do so or do so with greatly reduced efficiency. Commensal species are those that reap a benefit from another species but do not provide any benefit in return (Boucher et al. 1982).

Because obligate mutualists are more reliant on the resources provided by their partner species than facultative or commensal species, they suffer greater fitness reductions in the absence of partner species. This pattern has been demonstrated in multiple systems. For example, the pollen of obligate animal-pollinated plants has traits that allow attachment to animals but lacks traits that readily allow transport by wind (Stebbins 1970). Many plants host ants that

defend the plants from herbivores. When ants are excluded, plant species that are obligately guarded by ants suffer greater foliage loss than those facultatively guarded (Fiala et al. 1994, Heil et al. 2001, Rosumek et al. 2009). A similar mutualism occurs between ants and aphids, in which ants provide protection and aphids provide a sugar solution (Stadler and Dixon 2005). Once again, in the absence of ants, aphid species obligately guarded by ants suffer greater decreases in fecundity than those that are facultatively guarded (Stadler et al. 2002). There are facultative and obligate mutualists among corals that host mutualistic zooxanthellae (endosymbiotic dinoflagellates). Obligate coral species “bleach” and die if their zooxanthellae symbionts are expelled and not resequestered (Császár et al. 2010). In contrast, corals facultatively associated with zooxanthellae regularly expel and live for long periods without zooxanthellae (Dimond and Carrington 2008).

In most cases, it can be assumed that obligate mutualist species evolve from either commensal or facultative mutualists, with the exception of those that have obligate mutualist ancestors (Pellmyr et al. 1996) and those that are derived from parasitic symbionts (Aanen and Hoekstra 2007). Both obligate and facultative mutualist species are likely to exhibit traits (behavioral, morphological, or physiological) that allow participation in mutualisms. However, some traits are harbored exclusively by obligate mutualists, for example, plants that are facultatively guarded by ants, with few exceptions, only provide extra floral nectaries for their ant associates, in contrast with plants that are obligately defended and provide their ant associates with shelter in the form of hollow stems or thorns and protein and lipid rich food bodies. (Bronstein et al. 2006). Such traits may have evolved through two mechanisms. First, they may have been gained during a transition to obligate mutualism, perhaps through coevolution (Thompson 1994, Jousset et al. 2003). Alternatively, such traits may allow only a

certain subset of species already harboring them to evolve obligate mutualism, i.e., these traits pre-adapt certain species to obligate mutualism (Pellmyr et al. 1996). Thus, by comparing differences between obligate and facultative mutualists and related non-mutualists, we can start to explain the transitional pathways in traits and perhaps understand the mechanisms regulating the evolution of mutualism. Such is the goal of this study.

I focused on a protective and sheltering mutualism between an alpheid shrimp and three gobiid fishes in the Bahamas (Karplus 1987). The shrimp constructs a burrow in the sand, which is used by the mutualist partners in avoiding predators. Mutualist gobies are themselves incapable of burrowing and are quickly eaten when shrimp are absent, especially when no other shelter is available (Thompson 2005). Mutualist shrimp have poor vision, and consequently are vulnerable when they emerge from their burrows when maintaining the burrow or foraging in the periphery of their burrow entrance (Karplus 1987). However, gobies remain at burrow entrances and warn their shrimp partners when predators are present, allowing safe emergence (Karplus 1987). Warning takes two forms. First, a goby may rapidly flutter its caudal fin on the antennae of the shrimp. Once a predator is very near the burrow entrance, a second warning signal is used. This signal is a rapid head-first retreat into the burrow, which is more likely a self-preserving than warning behavior, but nonetheless at least serves as a warning to shrimp to retreat into the burrow (Karplus et al. 1972, Preston 1978, Karplus 1979, 1987). Shrimp without partners do not emerge from their burrows and suffer a growth decrement (Thompson 2003).

Over 120 gobiid species, within 12 genera, and 20 alpheid species, all within the genus *Alpheus*, participate in this mutualism (Thacker et al. 2011). Most of these associations occur in the Indo-Pacific. Interestingly, most gobies have been described as obligate associates with shrimp and exhibit the behaviors described above. In contrast, gobies facultatively associated

with shrimp rarely warn shrimp with caudal fin fluttering. However, shrimp use head-first retreats of these gobies as indication of the presence of predators. Gobies in obligate association with shrimp are within two distinct clades in the Indo-Pacific (Thacker et al. 2011), and one species, *Nes longus* (Nichols), in the western Atlantic (Karplus 1992, Ruber et al. 2003). Gobies in facultative association with shrimp include one species described in Japan (Yanagisawa 1978) and three described in the western Atlantic (Wayman 1973, Weiler 1976, Karplus 1992, Randall et al. 2005, Kramer et al. 2009).

The present study was conducted in the western Atlantic and focused on three species of goby: (1) the obligate shrimp-associate *N. longus*, (2) *Ctenogobius saepepallens* (Gilbert & Randall), which is one of the three gobies whose association with shrimp is described as facultative, and (3) *Coryphopterus glaucofraenum* (Gill), which I characterize here as a commensal species (Karplus 1992, Randall et al. 2005, Kramer et al. 2009). All three use the burrows of *Alpheus floridanus* (Kingsley), although each associates with the shrimp in a different way. *N. longus* are almost always associated with shrimp burrows, *C. saepepallens* less so, and *C. glaucofraenum* even less. *N. longus* often uses both warnings, *C. saepepallens* rarely uses caudal fin warnings, and *C. glaucofraenum* has never been reported using either. None of the three gobies are sister species and none are closely related to any other shrimp-associated gobies (Thacker and Cole 2002, Ruber et al. 2003, Thacker 2003, Pezold and Buth 2004).

I conducted a set of experiments on *N. longus*, *C. saepepallens*, and *C. glaucofraenum* to test four predictions on the distinction between obligate and facultative mutualists and commensal species. Shrimp burrows are one of many different resources that a goby can use when avoiding predators. Other shelters are sometimes available, such as live coral, coral rubble, sea grasses, mollusc shells, and burrows of other crustaceans. The first prediction is that

preference for shrimp burrows versus other shelters will be ordered *N. longus* > *C. saepepallens* > *C. glaucofraenum*.

Gobies can budget different amounts of time toward remaining at burrow entrances guarding shrimp partners as opposed to foraging in the vicinity of the burrow. Time spent at the burrow entrance is the resource allocated by the gobies to shrimp. By spending more time at a burrow entrance, a goby allows its shrimp partner more opportunity to safely emerge from the burrow (Thompson 2003). The second prediction is that time spent guarding shrimp will be ordered *N. longus* > *C. saepepallens* > *C. glaucofraenum*.

Gobies can switch between individual different shrimp partners on different time scales. The third prediction is that the time spent with an individual shrimp partner, before switching to another, will be ordered *N. longus* > *C. saepepallens* > *C. glaucofraenum*.

Species are likely to be differentially useful to their mutualist partner species. A goby is useful to a shrimp by allowing the shrimp safe emergence. However, it is unlikely that shrimp will have similar patterns of emergence with all three goby species because of differences in behavior of the gobies, which likely confer different degrees of protection of the shrimp from predators. The fourth prediction is that the number of shrimp emergences with a focal goby present will be ordered *N. longus* > *C. saepepallens* > *C. glaucofraenum*.

Each of these predictions was upheld and there was a clear gradient from commensal to facultative mutualist to obligate mutualist for each. There was a slight deviation from the third prediction in that I found that *C. saepepallens* and *C. glaucofraenum* remained at individual shrimp burrows for a similar duration, but *N. longus* remained with individual shrimp partners much longer. Below, I discuss these differences to draw two main conclusions: (1) increasing

reliance on a mutualist partner species is matched with increasing preference for the resource the mutualist partner provides over other resources and (2) increasing preference for the resource provided by a mutualist partner necessitates provisioning better quality and quantity of resources to the mutualist partner.

1.2 Methods

All experiments and observations were conducted at the Perry Institute for Marine Sciences located at Lee Stocking Island, Exuma, Bahamas. Experiments and observations were carried out during the summers of 2008 and 2009. I focused on the presumed obligate goby *Nes longus*, the facultative mutualist *C. saepepallens*, and the presumed commensal *C. glaucofraenum*. All three species are abundant in the shallow waters around Exuma. A second described facultative goby, *Bathygobius curacao* (Metzelaar), was present but very rare and was omitted from the study.

For most of the studies, I used two shallow field sites dominated by seagrasses: Normans (23°45'35.64"N, 76° 7'59.64"W) and Woobie (23°49'4.55"N, 76°11'17.43"W). The two field sites were quite different. *A. floridanus* burrows, other types of burrows, and seagrasses were more abundant at site Normans (Appendix). Predatory jacks, *Carangoides ruber* (Bloch) and *Carangoides bartholomaei* (Cuvier), and lizard fish, *Synodus intermedius* (Spix and Agassiz) occurred at both sites. *C. glaucofraenum* was absent at the site Woobie but was present on a nearby reef.

At each site, three 35-m transects were run, with 1-m² quadrats every 5 m (n=21 quadrats per site). The number of *A. floridanus* burrows, other burrows, blades of turtle grass (*Thalassia*

testudinum Banks ex König), manatee grass (*Syringodium filiforme* Kuetz), and percent debris cover (algae, dead uprooted seagrass, etc.) was recorded for each quadrat.

Resource preference by gobies

A laboratory experiment was designed to test whether gobies find and preferentially use the burrows of *A. floridanus* rather than other shelters. Pairs of artificial burrows of white polyvinyl chloride (PVC) pipe measuring approximately 20 cm long, with an inner diameter of 2.5 cm, were placed at opposite ends of 190-liter aquaria. These dimensions are comparable to the size of actual *A. floridanus* burrows (Dworschak and Ott 1993). One end of each PVC pipe was dug into sand at the bottom of the aquarium so that the open end barely protruded from the sand. A shrimp was placed in one burrow and the other was left empty. These artificial burrows are suitable because *A. floridanus* and the three goby species assume natural behaviors within. For example, *A. floridanus* will excavate sand from within the burrow and *N. longus* will remain at the entrance guarding the shrimp.

Forty-five different goby individuals from each of the three species (*N. longus*, *C. saepepallens*, and *C. glaucofraenum*) collected from site Normans were placed individually midway between the paired burrows. I checked the aquaria approximately every five minutes until the goby was present in one of the two burrows. I analyzed the data using a 3×2 G-test (three gobies species with two choices each). Comparisons of each goby species to the null expectation (shrimp burrow chosen 50% of the time) were conducted using a goodness-of-fit test.

Resource contribution by gobies

Quantitative field surveys were conducted using the method of Karplus (1992). Twenty gobies of each of the three species (*N. longus*, *C. saepepallens*, and *C. glaucofraenum*) were observed at both site Normans and site Woobie. Gobies were chosen haphazardly for timed observations. The first goby to be spotted was observed first, and the end of each observation period, the next goby to be spotted was observed unless it had been observed previously. Due to time constraints, these observations had to be carried out during several dives. However, I conducted observations in different areas (of the two sites) during different dives, to reduce the likelihood of observing the same goby during two different dives. Before gobies were observed, I allowed a five-minute period during which the goby could acclimate to the presence of a SCUBA diver and resume normal behavior (Karplus 1992). Each goby was then observed for ten minutes, during which I continually estimated its spatial position using a standard spatial reference system based on distance from the shrimp burrow opening (Fig. 1.1). The time a goby spent in each position (A through Y in Fig. 1.1) was recorded.

There were eleven dependent variables for each goby, i.e. time spent in each of eleven positions (Fig. 1.1). Because of the data structure, a multivariate test was most appropriate. However, there were two properties that made the data unsuitable for parametric multivariate analysis of variance (MANOVA): (1) the dependent variables were non-independent as time spent in one position detracted from time that could be spent at others, and (2) the data were non-normal due to a large number of zeros, because the gobies did not use all eleven positions during the 10-minute observation periods. Because of these limitations, I used a permutation MANOVA, which assumes only that observations (individual gobies) were independent (Anderson 2001). This analysis was conducted using the “adonis” function (vegan package) in

R 2.9.2. A two-way test was conducted using species and site as independent variables. It was found that site had no effect on time spent in different locations but species did. Thus, the data from both sites were combined, and three pairwise comparisons between the three species were conducted with the use of a Bonferroni probability correction (P-value of 0.017 gives an alpha level of 0.05).

Partner fidelity of gobies

In situ field observations were used to estimate the time each focal goby spent at an individual shrimp burrow before moving to another. However, a 10-minute observation period is probably too short to observe such movements of *N. longus* between shelters. *C. saepepallens* and *C. glaucofraenum* move regularly during 10-minute observation periods, but only one *N. longus* individual of 40 observed switched burrows during the 10-minute period. To gain a better estimate of *N. longus* movements, I tagged 15 individuals at site Normans in July 2009 and 15 in July 2010, and recorded the burrow in which each individual was located daily. Individuals were tagged with visible elastomer tags (Northwest Marine Technologies, Inc.), which have a minimal impact on growth, mortality, and susceptibility of small coral reef fishes to predators (Beukers et al. 1995, Frederick 1997, Malone et al. 1999). For the analysis, I included only individuals that were located on two days or more. I converted these data (time in days per burrow) into time in minutes per burrow. A one-way ANOVA and pairwise comparisons were used to analyze the data.

Benefit to shrimp partners

During the *in situ* observations described above, I also recorded the number of times a shrimp exited the burrow while the focal goby was present at the burrow entrance. For each goby observed, I calculated the number of times shrimp emerged from a shelter per unit time that the focal goby spent in position A, nearest the burrow entrance (Fig. 1.1). I conducted a two-way ANOVA with species and site as independent variables and shrimp emergence as the dependent variable, using R 2.9.2.

Night vs. day comparison of activity

I placed a video camera at the entrance of *A. floridanus* burrows to compare diurnal and nocturnal activity levels of *A. floridanus* and the three goby species. Video was also used to record the behavior of the three goby species with SCUBA divers absent to allow a qualitative comparison of goby behavior with SCUBA divers present and absent. The video camera was mounted on a stand and positioned approximately 40 cm from an *A. floridanus* burrow, such that the burrow and 20 cm area around the burrow was in the video frame. The stand was positioned before each daytime video and then kept in the same position for the nighttime video. Thus, nocturnal and diurnal activity at an individual burrow could be compared. For nighttime videos, a dive light was also mounted on the same stand. Six one-hour videos were taken (three night and three day). Daytime videos were taken between 1500 and 1700 hrs and night videos between 2200 and 2400 hrs. Night videos were initiated well after the last light of day, and the dive light was not switched on until the video was started. The videos were taken under the dock at Lee Stocking Island (23°46'21.73" N, 76°06'25.85" W). For each video, I tallied the total time in

which an individual of each goby species was present in the video frame, as well as the number of times that an *A. floridanus* individual emerged from the burrow.

I qualitatively compared behavior of gobies with SCUBA divers present and absent. Unfortunately, I could not do a direct quantitative comparison for two reasons. First, data from the section “*resource contribution by gobies*” is comprised of 10-minute observations of gobies, but in videos, gobies move in and out of the frame much faster than 10 minutes. Second, videos did not have the same spatial coverage as direct observations as SCUBA divers and did not include the positions I, X, and Y (Fig. 1.1).

1.3 Results

Resource preference of gobies

Preference for the artificial shrimp burrow (i.e., PVC pipe) versus the non-shrimp burrow was very different between goby species. The overall 3×2 test was significant ($G_{adjusted} = 21.43$, $df = 2$, $P < 0.001$), revealing that there were difference among gobies. *N. longus* was associated with shrimp significantly more often from the 50% null expectation ($G_{adjusted} = 13.13$, $df = 1$, $P < 0.001$), *C. glaucofraenum* was associated with the shrimp less often than expected ($G_{adjusted} = 0.46$, $df = 1$, $P = 0.022$), and *C. saepepallens* was not different from the null expectation, indicating indifference to shrimp ($G_{adjusted} = 0.463$, $df = 1$, $P = 0.496$).

Resource contributions by gobies

Field site had no effect on time allocation, but the three goby species allocated different amounts of time to different positions in the reference grid (Fig. 1.1, 2; Table 1.1). Among positions, the majority of the variation was accounted for by position A (49.5% of the variation) followed by X (16.5%), I (7.5%), E (6.1%), C (6.1%), and Y (5.5%; Fig. 1.2). The rest in total accounted for 8% of the variation. Among gobies, *N. longus* accounted for 49.5% of the variation, *C. glaucofraenum* 26.0% and *C. saepepallens* 24.5%. Multiple comparisons demonstrated statistically significant differences between *N. longus* and *C. saepepallens* ($F_{1,79} = 31.74$, $P < 0.001$), *N. longus* and *C. glaucofraenum* ($F_{1,59} = 50.38$, $P < 0.001$), and *C. saepepallens* and *C. glaucofraenum* ($F_{1,59} = 3.40$, $P < 0.01$).

Partner fidelity of gobies

The three gobies spent different durations at burrow entrances ($F_{2,70} = 29.35$, $P < 0.001$). *N. longus* spent 2.52 ± 1.41 days (95% CI; $n = 13$), *C. saepepallens* 5.48 ± 0.98 minutes ($n = 40$), and *C. glaucofraenum* 6.26 ± 1.11 minutes ($n = 20$). Pairwise comparisons revealed that *N. longus* was different from *C. saepepallens* ($F_{1,51} = 39.27$, $P < 0.001$) and *C. glaucofraenum* ($F_{1,31} = 19.16$, $P < 0.001$), but *C. saepepallens* and *C. glaucofraenum* were not different ($F_{1,58} = 0.732$, $P = 0.396$).

Benefit to shrimp partners

Shrimp emerged significantly more often with *N. longus* present (2.26 ± 0.41 times per minute) than *C. saepepallens* (0.51 ± 0.40 times per minute; Table 1.2), but they never emerged when *C. glaucofraenum* were present. Shrimp were never found emerging without a goby at the burrow entrance.

Night vs. day comparison of activity

All three gobies were more active during the day than at night, despite the inherent bias of artificial lighting at night. In all six videos, *N. longus*, *C. saepepallens*, and *C. glaucofraenum* individuals were present 68.50, 26.30, and 3.48% of the time at day and 1.11, 6.04, and 0.26% of the time at night, respectively. *A. floridanus* emerged 123 times during the day versus zero times at night.

The behavior of each of the three species is qualitatively similar during observations using video cameras (no SCUBA diver present) and with a SCUBA diver present. In both situations, *N. longus* individuals remained at burrow entrances warning shrimp when predators approached, and *C. saepepallens* and *C. glaucofraenum* did not remain at burrow entrances for long periods of time, but rather roamed from burrow to burrow. Thus, observations made by SCUBA divers in this study accurately characterized the behavior of these species.

1.4 Discussion

All aspects of association shrimp varied among the three goby species in the directions predicted. Both preference for shrimp burrows versus other shelters (prediction one) and time spent at burrow entrances (prediction two) were ordered *N. longus* > *C. saepepallens* > *C. glaucofraenum*. The number of times shrimp emerged with each goby species present was ordered *N. longus* > *C. saepepallens* > *C. glaucofraenum* (prediction four). Time spent at individual burrows before switching to another was ordered *N. longus* >>> *C. saepepallens* = *C. glaucofraenum* (prediction three). Goby behavior was similar with and without SCUBA divers present. Activity of all three gobies and the shrimp ceased or was reduced at night.

Resource preference by mutualists

Often, individuals of a mutualist species have several means of gaining a resource. Resource specialization for a mutualist species would be characterized by exclusive use of the resource provided by the partner species. For example, plants obligately pollinated by animals are more specialized than those pollinated by both animals and the wind (Stebbins 1970). Both fig wasps and yucca moths are obligate pollinators and individual species have mouth parts specialized to their host fig or yucca plants, respectively (Pellmyr and Krenn 2002, Cook et al. 2004). González-Teuber & Heil (2009) found that two ant species that obligately defend and live within plants prefer nectar with an amino acid composition most similar to that of their host plant. In contrast, two ant species in facultative association with plants had no preference. Obligate pollinators are strongly attracted to defensive floral scent compounds of the plants they

visit, whereas facultative pollinators are repelled by the same compounds (Junker and Blüthgen 2010).

Specialization is likely the result of a coevolved history, which is more likely to occur between obligate mutualists than facultative mutualists (Thompson 1994). Some of the most highly cited examples of coevolution are those between pollinators and the plants they pollinate, for example, the orchid *Angraecum sesquipedale*, which has a long corolla, and its pollinator the hawk moth, *Xanthopan morgani praedicta*, which has an extra long proboscis as was famously predicted by Darwin (Darwin 1862, Padian 2008).

Regarding resource preference of the three goby species, *N. longus* preferred *A. floridanus* burrows, *C. saepepallens* had no preference, and *C. glaucofraenum* preferred shelters not constructed by *A. floridanus*. Interestingly, *C. glaucofraenum* avoided shelters with *A. floridanus* in the lab, but used their burrows at the field site Normans. At site Woobie, in which *A. floridanus* burrows were the majority of shelters (see appendix), *C. glaucofraenum* is absent, but is present on a nearby reef. These findings may be explained by one additional observation.

A. floridanus burrows are as much as 30 cm deep with 70 cm of horizontal extension (Dworschak and Ott 1993). However, my field observations suggest that *C. glaucofraenum*, and to a lesser extent, *C. saepepallens*, do not venture deep into *A. floridanus* burrows and may rarely come into contact with resident shrimp. The easiest way to collect these gobies is to scare a targeted goby into a burrow and dig a flat object into the sand, blocking access to the part of the burrow 15 cm or deeper. Thus, a target goby that has not entered deeper than 15 cm is isolated from the deeper part of the burrow and easily extracted. *C. glaucofraenum* individuals do not enter very far into the burrow, making them easier to extract. *C. saepepallens* individuals are

more difficult to extract and *N. longus* even more difficult. At sites such as Normans, which has many potential shelters not constructed by *A. floridanus*, *C. glaucofraenum* may use *A. floridanus* burrows accidentally and never contact shrimp. *C. glaucofraenum* probably avoids sites such as Woobie, where all potential shelters contain resident *A. floridanus*.

These findings clarify an important component of the evolution of mutualism. A transition from commensal to facultative mutualism involves a switch from exclusive (or at least primary) usage of a resource gained outside the mutualism to use of both resources gained outside and through the mutualism. Further, a transition from facultative to obligate mutualism involves a switch from use of both resource types to exclusive (or primary) usage of the resource gained through the mutualism. It might be expected that shrimp burrows would offer better protection than other shelters due to their depth (Dworschak and Ott 1993), and consequently, all goby species would prefer shelter-containing shrimp. The data suggest otherwise, and there are multiple possible explanations for why this may be the case, three of which seem most likely. First, observations suggest that *N. longus* may exclude *C. saepepallens* and *C. glaucofraenum* from burrows such that the later two cannot evolve a preference for shrimp burrows (Randall et al. 2005). Second, there may be a cost associated with using shrimp burrows. Remaining at the entrance of an individual shrimp burrow, such as *N. longus* does, may be costly because foraging is reduced to the small area outside the burrow entrance. Lastly, shrimp may be able to distinguish the three gobies and aggressively exclude individuals based on species identity.

The cost of partner absence and its effect on resource provisioning

Mutualism is a trading of resources, which are more beneficial to the recipient than donor. It is costly to produce a resource to be donated, but the benefit associated with receiving a resource will outweigh this cost for mutualism to evolve (Connor 1995). When an individual of a mutualist species cannot form an association with an individual of the partner species, it suffers a different cost, which is partner absence. For obligate mutualists, the cost of partner absence is death or a major fitness reduction. In contrast, facultative mutualists and commensal species suffer a smaller fitness reduction, which may be compensated by use of alternative resources. An individual can reduce the likelihood of paying the cost of partner absence by creating a resource of higher quality or quantity. By doing so, an individual will attract more partners, ensure the survival of its partner, and provide individual partners with incentive to continue the association (Bull and Rice 1991, Hanley et al. 2008, Heil et al. 2009).

Roughgarden (1975) modeled the cost of partner absence, and found it likely to affect the quality of resources provisioned. In anemone-fish mutualism, an anemone provides a fish with a shelter and the fish provides several resources. Each of several anemone fish species has a specific host anemone species. Individuals of fish species with a common host anemone only provide food to their host anemone. In contrast, individuals of an anemone fish species with a rare host anemone provision food, and additionally excavate crevices for their host anemone to inhabit. The second fish has a greater cost of partner absence than the first, because the host anemone is more difficult to locate because of its rarity. Therefore, individuals of the second fish species must provide a higher quality resource (food plus crevice excavation) to ensure the survival of their hosts.

A similar result has been found in other mutualism systems. Plants that are obligately pollinated by animals provide rewards of greater quality than do plants that are facultatively pollinated by animals; consequently, obligately pollinated plants receive more pollinator visits (Hanley et al. 2008). Plants that are obligately guarded by ants offer their ant guests resources not provided by facultatively guarded plants (shelter and food bodies), and are consequently inhabited by ants more often (Bronstein et al. 2006, Heil et al. 2009).

For gobies, the cost of shrimp absence is increased vulnerability to predators while the goby has no shelter. A means of reducing the cost of shrimp absence would be to remain with shrimp for longer periods. *N. longus* individuals remained with individual shrimp for much longer periods than did *C. saepepallens* or *C. glaucofraenum*. This finding implies that the cost of shrimp absence is much greater for *N. longus* than the other two gobies. Not surprisingly, *N. longus* spends the most time at burrow entrances. By remaining at a burrow entrance, a goby allows its shrimp safe emergence and ensures the survival of the shrimp. Not surprisingly, *A. floridanus* individuals emerged most often when paired with *N. longus*. Thus, *N. longus* is of most use to the shrimp. It is possible that the shrimp emerges more often with *N. longus* present than the other goby species simply because *N. longus* spends more time in position A (Fig. 1.1). However, the behavior of the three gobies may have an additional effect on shrimp emergence. The shrimp may be able to discriminate between the three goby species using chemical, physical, or behavioral cues. For example, in the Red Sea, where there is a much larger group of mutualist gobiid fishes and alpheid shrimps, several but not all shrimp species will associate only with a single preferred goby species (Karplus 1981, Karplus et al. 1981). It is possible that *N. longus* and *A. floridanus* have such a partner-specific association.

These findings lend support to the supposition that obligate mutualists must provide higher quality or quantity resources than facultative mutualists or commensal species. Findings here, and on other mutualistic systems, indicate an important component of the evolutionary transition from commensal to facultative to obligate mutualism. As a species becomes increasingly dependent on its mutualist partner species, it must provide better quality or quantity resources to reduce the likelihood of costly partner absence. Consequently, dependent species incur an increasing cost associated with resource provision. If a species is not able to evolve means for coping with this cost, then it may be constrained to a less dependent association than those species that can overcome this cost (Bronstein 2001).

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Table 1.1 Two-way permutation MANOVA with the dependent variable time spent in each the 11 different positions of the reference grid (Fig. 1.1). Data includes all three goby species at both field sites.

Source	df	SS	MS	F	P-value
Species	2	8.12	4.060	22.29	< 0.001
Site	1	0.095	0.095	0.526	0.728
Species*Site	1	0.187	0.187	1.027	0.360
Residuals	95	17.30	0.182		
Total	99	25.70			

Table 1.2 Results of a two-way ANOVA with species and location as independent variables and shrimp emergence times from a burrow with the focal goby in position A as the dependent variable.

Source	df	SS	MS	F	P-value
Species	2	0.019	0.009	24.27	< 0.001
Location	1	0.0001	0.0001	0.251	0.618
Species*Location	1	0.0001	0.0001	0.167	0.684
Residuals	74	0.029	0.0004		
Total	78	0.049			

Figure 1.1 Positions used to quantify goby location and burrow use. On the left is a typical cone shaped *A. floridanus* burrow (positions A through Y). Position A is the area into which *A. floridanus* emerges from the burrow. On the right is represented any of various shelters alternative to *A. floridanus* burrows (shells, coral rubble, etc). For both shrimp burrows and alternative shelters, the center (black) is the entrance to the shelter/burrow. For the inner ring (positions A through D and X) are within 10 cm of the entrance. Positions E through H and Y are between 10 and 30 cm of the entrance. Position I is beyond 30 cm of any entrance. Modified from Karplus (1992).

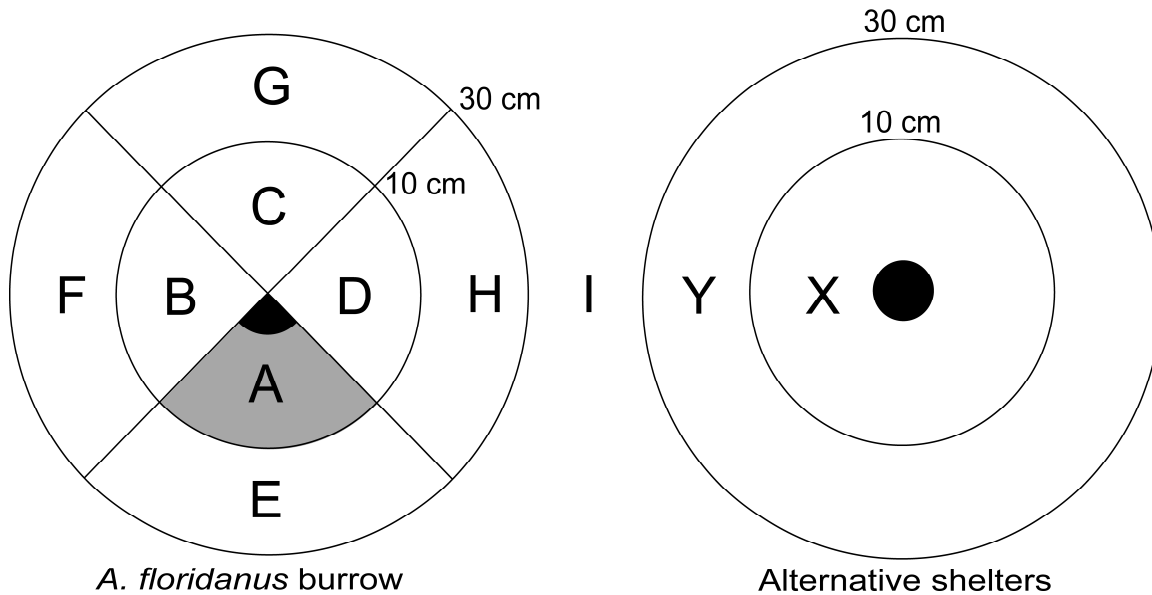
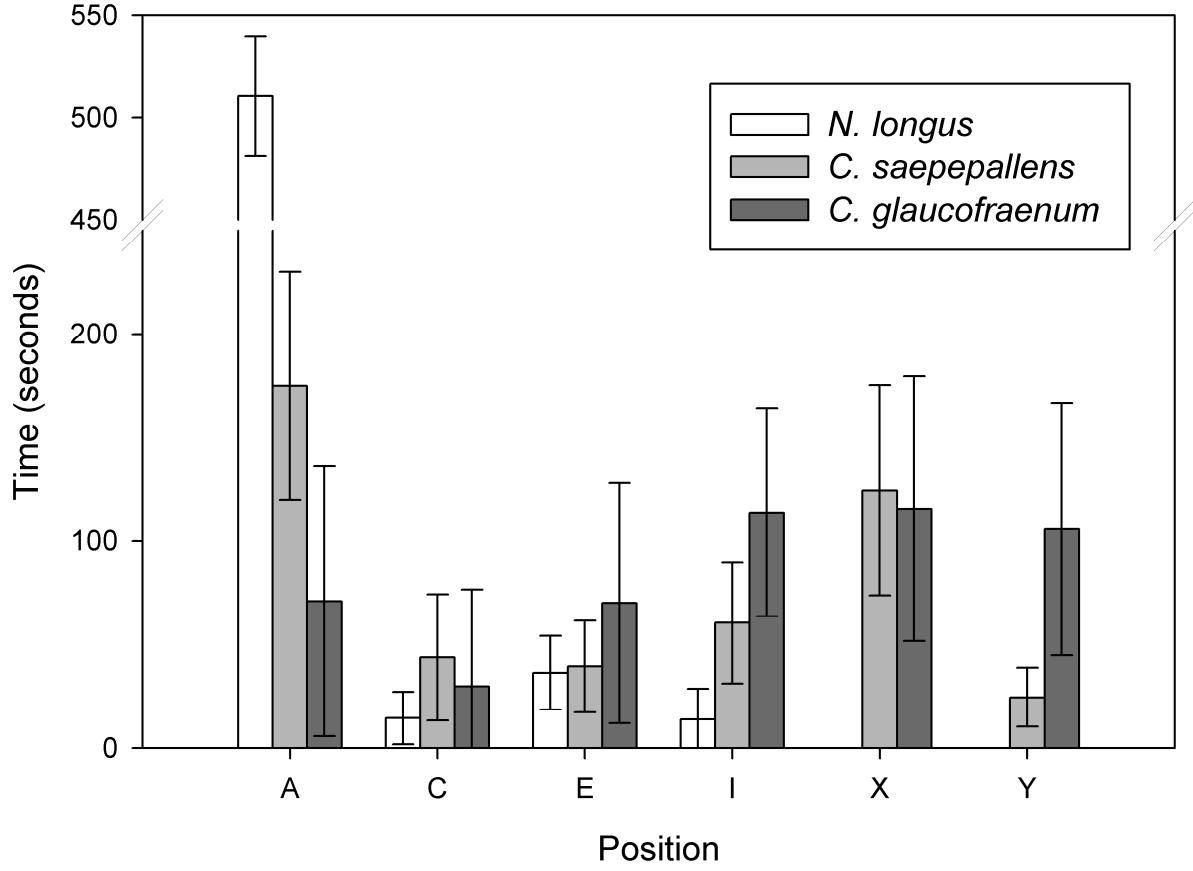


Figure 1.2 Mean time (\pm 95% CI) gobies spent in each position. For *N. longus* and *C. saepepallens*, data are from both field sites. Note the break on the Y axis between 250 and 450 seconds. Only positions that had major contributions to the total variation are shown.



Chapter 2. The benefit of mutualism to obligate and facultative mutualists in a shrimp-goby association

Abstract. Mutualist species benefit from resources provided by their partner species. Obligate mutualists are predicted to incur a greater benefit from these resources than facultative mutualists. Individual poor-sighted shrimp (*Alpheus floridanus*) construct burrows that are shared with individual gobiid fishes that warn shrimp when emergence from burrows is unsafe. The benefit to gobies is shelter from predation. I compared predator avoidance ability of obligate (*Nes longus*) and facultative (*Ctenogobius saepepallens*) shrimp-associated gobies with access to a shell, a shrimp burrow, or no shelter. Predator avoidance effectiveness was ranked $N. longus$ (no shelter) = $C. saepepallens$ (no shelter) < $N. longus$ (shell) = $C. saepepallens$ (shell) = $C. saepepallens$ (shrimp burrow) < $N. longus$ (shrimp burrow). Thus, *N. longus* benefits more from mutualism with shrimp than *C. saepepallens*. *N. longus* has four behaviors related to predator avoidance when shrimp burrows are present: (1) *N. longus* always dives into the nearest shrimp burrow when confronted with predators. (2) *N. longus* has a longer flight initiation distance than *C. saepepallens*. (3) After taking refuge, *N. longus* re-emerges from burrows after a longer time than *C. saepepallens*. (4) *N. longus* remains closer to shelter than *C. saepepallens*. The latter three behaviors are likely detrimental for foraging as they limit foraging range and detract from time spent outside shrimp burrows foraging. Thus, it is likely that the two goby species are equally dependent on shelter, but *C. saepepallens* may have foraging requirements that constrain it to a less beneficial association with *A. floridanus* than *N. longus*.

2.1 Introduction

Mutualism is an interspecific interaction in which both species obtain a net benefit (Boucher et al. 1982). The benefit of receiving a resource from a partner outweighs the cost of provisioning the partner with a resource, sometimes because the resource provisioned may be a by-product and of little value to the producer (Connor 1995). Mutualist species vary in the degree to which they rely on the resources provided by their partner species. Obligate mutualists are strongly reliant on these resources and cannot exist without their partners (Boucher et al. 1982). In contrast, facultative mutualists are not reliant, because they often can gain these resources through means other than their partners. For example, many fishes facultatively clean larger fishes, but can also persist on a diet of non-parasitic invertebrates (Côté 2000, White et al. 2007). I report an experimental study of the protective mutualism between an alpheid shrimp and two gobiid fishes (Karplus 1987, Karplus & Thompson 2011). The shrimp constructs a burrow in sand that it co-habits with an individual goby. Both use the burrow as refuge from predators. Gobies without access to shrimp burrows are quickly eaten when predators are present (Thompson 2005). Shrimp have poor vision and are vulnerable to predators when outside their burrows foraging or excavating their burrow entrances (Thompson 2003). Gobies have good vision and shrimp use two behaviors of gobies as indication of approaching predators. The first is a fluttering of the caudal fin on the antennae of the shrimp. The second is a head-first dive by the goby into the burrow (Preston 1978). Shrimp without gobies rarely leave their burrows and suffer a growth decrement (Thompson 2003).

Over 120 gobiid species in 12 genera, and 20 alpheid species all in the genus *Alpheus*, participate in this mutualism (Karplus & Thompson 2011). Most of these gobies follow the behaviors described above and are considered obligate mutualists (Karplus & Thompson 2011).

Obligate mutualist gobies comprise two clades in the Pacific and one species in the Atlantic (*Nes longus*). These two clades and *N. longus* are polyphyletic and are separately nested within non-shrimp-associated gobies (Rüber et al. 2003, Thacker et al. 2011). Only four gobies have been described as facultative mutualists with shrimp, and they rarely provide the caudal fin flutter warning: one species in Japan (Yanagisawa 1978, 1984) and three in the Western Atlantic and Caribbean (Wayman 1973, Weiler 1976, Karplus 1992, Randall et al. 2005, Kramer et al. 2009, Chapter 1).

I previously compared the behaviors of the obligate mutualist goby, *N. longus*, to one of the Atlantic facultative mutualist gobies, *Ctenogobius saepepallens*. Both associate with the shrimp *Alpheus floridanus*. These gobies are not sister species to each other or any other shrimp-associated gobies (Rüber et al. 2003, Thacker 2003). *N. longus* has stronger preference for *A. floridanus* burrows versus other shelters, spends more time communicating with *A. floridanus*, and remains with individual *A. floridanus* partners for much longer periods than *C. saepepallens* (Chapter 1).

Here, I compared *N. longus* and *C. saepepallens* for the benefit of mutualism. For shrimp-associated gobies, the benefit of mutualism is increased predator avoidance while inhabiting shrimp burrows rather than other shelters. I predicted the benefit of mutualism to be greater for *N. longus* than *C. saepepallens*. I examined predator avoidance by gobies with access to a shrimp burrow, a shell, or no shelter. In addition, I quantified behaviors relating to predator avoidance.

2.2 Methods

Study sites

All the experiments and observations were carried out at the Perry Institute for Marine Sciences, Lee Stocking Island, Exuma, Bahamas during the summers of 2009-2011. I used two field sites: “Normans” (23°45'35.64"N, 76° 7'59.64"W), which was composed of bare sand with coral rubble and various seagrass species and “Woobie” (23°49'4.55"N, 76°11'17.43"W), which was entirely bare sand with shrimp burrows offering the only shelter. A quantitative description of these two sites is provided in Chapter 1. Individuals used in laboratory experiments were collected at the Normans site.

Avoidance of live predators

I conducted staged interactions between an individual predator and a *N. longus* or *C. saepepallens* individual with access to one of three shelter types: an artificial shrimp burrow with a resident shrimp, a conch shell, or no shelter. Trials were conducted in 190-l aquaria (119 X 30.5 X 48.5 cm) based on Abrahams and Kattenfeld (1997) and Mirza and Chivers (2000). The bottom of each aquarium was covered with sand 10 cm deep. Artificial shrimp burrows were made of PVC tubing 20 cm long with a 2.5-cm inner diameter. These PVC tubes were suitable surrogates for natural *A. floridanus* burrows for four reasons: (1) they were similar in diameter and orientation to natural *A. floridanus* burrows (Dworschak & Ott 1993); (2) *A. floridanus* readily entered tubes and excavated sand from within them; (3) both *N. longus* and *C.*

saepepallens readily entered tubes and *N. longus* guarded resident shrimp within; and (4) once a goby entered a PVC tube, it was isolated from predators as with natural *A. floridanus* burrows.

Cephalopholis fulva (coney grouper) was used as the live predator. *C. fulva* is a common generalist piscivore on Western Atlantic reefs. Grouper total length ranged from 12 to 22 cm, and had no effect on trial outcome (successful or unsuccessful capture of the goby; logistic regression: $X^2 = 0.88$, $df = 1$, $P = 0.35$). Groupers were housed individually in aquaria and allowed to acclimate for five days. During each day that trials were conducted, each grouper was used for only six trials to prevent satiation. Groupers always targeted gobies during trials.

A 10-minute acclimation period preceded each trial, during which the goby could find the shelter (if present) and was separated from the grouper by a clear divider. The grouper typically moved to the half of the aquarium with the goby as soon as the divider was removed. However, several did not. Thus, the 15-minute trial period started when the grouper swam midway across the aquarium to the half with the goby. In each trial, I recorded the time until the goby was captured. If the trial lasted the entire 15 minutes, I removed the goby and recorded the outcome as no capture.

Individual gobies were used only once for each trial. Forty trials were conducted ($2*3*40=240$ total trials) for each goby species (*N. longus* and *C. saepepallens*) and each shelter type (shrimp burrow, conch shell, or no shelter). Each grouper was used for two of each trial combination, i.e. two *N. longus**shrimp-burrow trials, two *N. longus**conch-shell trials, etc. Thus, each grouper was used for the same 12 trial combinations. For each grouper, the order of trials was randomized to control for learning.

Much of the time-until-capture data were right-censored, i.e. there were many trials in which the goby was never captured. Thus, time-until-capture data were analyzed using the subroutine “survdiff” (R 2.14.1, “survival” package), which compares survival curves using the log-rank Mantel-Haenszel test and accounts for right-censored data (Mantel 1966, Harrington & Fleming 1982). I compared all possible goby species-shelter combinations using the appropriate Bonferroni correction (15 tests, $df = 1$, $P = 0.003$ for 95% error rate).

Avoidance of a model predator

I staged interactions between a model predator (11-cm rubber fishing lure) and individuals of both goby species with access to an artificial shrimp burrow or no shelter. In a 190-l aquarium, I constructed a system of pulleys to move the model predator across the aquarium lengthwise using the force of a dropped 3-lb dive weight.

Very constant model-predator velocities were achieved during trials: mean \pm 95% confidence = 192.8 ± 5.5 cm/sec for no-shelter trials and 165 ± 7.4 cm/sec for shrimp-burrow trials. The model predator was likely slower for shrimp-burrow trials because these trials were completed last and corrosion of the pulleys likely slowed the model. Because model-predator speed was different for the two trial types, between-treatment comparisons were not made.

Gobies were tested individually in both no-shelter and shrimp-burrow trials. For each no-shelter trial, a goby was herded into the path of the model predator using an aquarium net. For each shrimp-burrow trial, the burrow was placed in the path of the model predator. The goby was allowed to enter the burrow and once it re-emerged, the trial was started. A total of 25 no-shelter and 10 shrimp-burrow trials were run for each goby species.

For no-shelter trials, the mean velocity of the model-predator was 5.8% faster for *C. saepepallens* than *N. longus* trials (mean \pm 95% confidence: 198.2 ± 10.1 cm/sec versus 187.2 ± 2.89 cm/sec, respectively). While this difference was slight, it was statistically significant (one-way ANOVA: $F_{1,47} = 4.04$, $P = 0.05$). There were five *C. saepepallens* trials in which the model speed was greater than one standard deviation above the grand mean (model speed of trials with both species). No *N. longus* trials were greater than one standard deviation above the grand mean. I separately ran each of the analyses below excluding these five *C. saepepallens* trials and found no difference in outcome. Thus, the results of analyses on goby flight performance were valid, despite slight differences in model-predator speed. There was no difference in model-predator speed during shrimp-burrow trials of *N. longus* and *C. saepepallens* (one-way ANOVA: $F_{1,18} = 0.052$, $P = 0.821$).

The reactions of gobies to the model predator were recorded from above using a Casio Elixim FH100 camera filming at 120 frames per second. Stills from these videos were examined using the image software GIMP (version 2.6.8) to calculate distances and velocities. Standards were used to calibrate the pixel-to-cm ratio. There were two independent variables: goby species and prior orientation (Fig. 2.1). I recorded four dependent variables for no-shelter trials: (1) use of a “C-type fast-start,” where a fish bends initially into a C-shape and darts away in response to a stimulus (Domenici & Blake 1997), (2) top and (3) average speed during flight (both in cm/sec and body lengths/sec), and (4) flight initiation distance (FID), which is the distance from a predator at which a prey reacts and begins flight (Ydenberg & Dill 1986). Prior orientation did not statistically affect any of these dependent variables, and had no interaction with goby species. Thus, the results only report the effect of goby species. For shrimp-burrow trials, FID was the only dependent variable and goby species the only independent variable.

In situ flight initiation distance, flight direction, re-emergence time, and distance-to-shelter

I conducted a set of *in situ* trials in the field in which I rapidly approached a focal goby and estimated FID, flight direction (retreat to the nearest burrow or a more distant one), and time until re-emergence from a burrow. I acted as a “predator” by rapidly swimming toward a focal goby from a distance of at least 3 m. Care was made not to observe any individual goby more than once. A total of 20 and 28 *N. longus* and 29 and 30 *C. saepepallens* were observed at the Normans and Woobie sites, respectively. I used a two-way ANOVA to analyze the effect of the independent variables site and goby species on the dependent variables FID and re-emergence time. To determine whether goby species affected the dependent variable flight direction, I first separately compared the two sites for each goby species using two 2×2 G-tests of independence. Site had no effect for either goby species. Thus, I combined the data for both sites and compared the two goby species using a 2×2 G-test of independence.

I also conducted *in situ* time budget observations of 20 gobies of each species at the Normans and Woobie sites following Karplus (1992). A complete description of the methodology and basic field data is in Chapter 1. During 10-min observation periods, I recorded the time a focal goby spent < 10 cm, 10 - 30 cm, and > 30 cm from any shelter (*A. floridanus* burrow, coral rubble, shell, etc.). There were three dependent variables corresponding to time spent in each of the three distance bins. Because time spent in one bin detracted from time spent in other bins, the dependent variables were not completely independent. Thus, the data were analyzed with a two-way permutation MANOVA using site and species as independent variables and time in each bin as the multivariate dependent variable (vegan package, R 2.14.1).

2.3 Results

Avoidance of live predators

Across all shelter types (no shelter, conch shell, shrimp burrow), *N. longus* had a greater survival time during staged predator interactions than did *C. saepepallens* (mean \pm 95% confidence: 497.2 ± 77.7 seconds versus 354.1 ± 74.5 seconds, respectively; $df = 1$, $X^2 = 7.2$, $P = 0.0074$). There were significant differences among individuals of both goby species for survival time among the three shelter types: no shelter (126.6 ± 62.0 seconds), conch shell (474.9 ± 93.0 seconds), and shrimp burrow (668.6 ± 85.0 seconds; $df = 2$, $X^2 = 86.4$, $P < 0.001$). *N. longus* and *C. saepepallens* had similar survival times during no-shelter and conch-shell trials, but *N. longus* had longer survival times than *C. saepepallens* during shrimp-burrow trials (Fig. 2.2).

Avoidance of a model predator

The two gobies had similar reactions to the model predator in no-shelter trials. *N. longus* and *C. saepepallens* used C-type fast-starts in 17 and 16 of 25 trials, respectively. The two gobies had similar top speed (mean \pm 95% confidence: *N. longus* = 39.0 ± 4.09 body-lengths/sec and *C. saepepallens* = 44.1 ± 3.64 body-lengths/sec; Table 2.1) and average speed (*N. longus* = 33.8 ± 3.16 body-lengths/sec and *C. saepepallens* = 37.8 ± 3.01 body-lengths/sec; Table 2.1). The two gobies had similar FIDs (*N. longus* = 7.15 ± 1.58 cm and *C. saepepallens* = 7.31 ± 1.81 cm; Table 2.1).

N. longus had a longer FID than *C. saepepallens* in shrimp-burrow trials (26.3 ± 6.23 cm versus 18.2 ± 4.45 cm, respectively $F_{1,18} = 4.30$, $P = 0.053$). Additionally, *N. longus* had a longer FID than *C. saepepallens in situ* (see below).

In situ flight initiation distance, flight direction, re-emergence time, and distance-to-shelter

N. longus had a longer *in situ* mean FID than *C. saepepallens* (Table 2.2, Fig. 2.3). *N. longus* had a longer mean FID at Woobie than Normans (Table 2.2, Fig. 2.3).

N. longus had a longer re-emergence time after taking refuge in a burrow than *C. saepepallens* (Table 2.2, Fig. 2.3). Site had no statistical effect on re-emergence time. *N. longus* always darted into the nearest burrow (48 out of 48 trials), in contrast to *C. saepepallens* (46/58 trials; $df = 1$, $G_{adj} = 10.77$, $P < 0.005$). Site had no effect on flight direction in *N. longus* ($df = 1$, $G_{adj} = 0$, $P = 1.0$) or *C. saepepallens* ($df = 1$, $G_{adj} = 0.978$, $P = 0.322$). *N. longus* individuals remained closer to shelter entrances than *C. saepepallens* (Fig. 2.4; Table 2.3). Similar results were reported by Kramer et al. (2009). Site had no effect on distance-to-shelter. *N. longus* were within 10 cm of a shelter entrance 87% of the time and *C. saepepallens* 65% of the time (Fig. 2.4). Neither goby was beyond 10 cm from shelter entrances very often (Fig. 2.4).

2.4 Discussion

The benefit of mutualism

Obligate mutualists should, by definition, benefit more from mutualism than facultative mutualists. For example, in associations between many plants and ants, the host plant provides

shelter and nectar and ants provide protection from herbivores (Heil & McKey 2003). When ants are excluded, obligate ant-associated *Macaranga* trees incur more leaf loss than facultative ant-associates (Fiala et al. 1994, Heil et al. 2001). A meta-analysis of 81 studies of ant-plant systems indicated the same difference between obligate and facultative mutualists (Rosumek et al. 2009). In another mutualism, aphids provide ants with honey dew and ants protect aphids from predators (Stadler et al. 2001, Stadler & Dixon 2005). Obligate ant-associated aphids suffer a fecundity loss when ants are excluded, but facultative associates do not (Stadler et al. 2002).

Given that obligate mutualists benefit more from mutualism than facultative mutualists, it is not surprising that many obligate mutualists have traits that reduce the probability of partner absence. Obligate mutualists often offer greater quality and quantity rewards than facultative mutualists. Better rewards increase the likelihood of establishment of association with mutualist partners. For example, obligately-pollinated plants offer pollen with a greater protein content and are visited by pollinators more frequently than facultatively-pollinated plants (Hanley et al. 2008). Ant-associated *Acacias* that provide more nectar, often host more ants and are more effectively protected from herbivores than *Acacias* that provide less nectar (Heil et al. 2009).

The two gobies in this study seem equally dependent on shelter, i.e. they avoided a live predator poorly when no shelter was present. This result is corroborated by the flight mechanics of the two gobies, which were similar when no shelter was present. The two gobies also avoided a live predator with equal effectiveness when a conch shell was present. However, *N. longus* more effectively avoided predators when a shrimp burrow was present than with a conch shell. In contrast, *C. saepepallens* avoided predators no more effectively when a shrimp burrow was present than with a conch shell.

Traits affecting the benefit of mutualism

The advantage of *N. longus* over *C. saepepallens* in avoidance of predators while using shrimp burrows is likely related to the four behaviors quantified: FID, flight direction, re-emergence time, and distance-to-shelter. For each of these behaviors, *N. longus* responded to the predator more effectively than *C. saepepallens*.

Flight direction is probably related to spatial map usage (Markel 1994, Dodd et al. 2000, Braithwaite 2006, Burt de Perera & Guilford 2008). *N. longus* always retreated to the nearest burrow, but *C. saepepallens* did not, which indicates that *N. longus* is better equipped in spatial-map usage, and therefore has an advantage in predator avoidance.

For the other three behaviors, *N. longus* also responded to the predator more effectively. But in the behaviors, there is likely a tradeoff between predator avoidance and foraging. It is unlikely that gobies often forage inside shrimp burrows. Two lines of evidence support this supposition. First, the abundance of meiofauna that *N. longus* and *C. saepepallens* consume, such as copepods (Randall et al. 2005), decreases with depth in the sediment (De Troch et al. 2008). Second, both species spend very little time inside the burrow during the day (mean \pm 95% confidence: *N. longus* = 2.6 ± 2.3 % total time and *C. saepepallens* = 6.8 ± 3.5 % total time; Chapter 1). If there were an abundant supply of food within burrows, gobies would not be expected to spend so much time outside of burrows when there is a high predation risk.

The decision to flee depends on the benefit of fleeing (predator avoidance) and costs of fleeing (ex. lost foraging opportunities; Ydenberg & Dill 1986). The longer FID of *N. longus* at burrow entrances may be better suited for predator avoidance, but the shorter FID of *C.*

saepepallens may be better suited for food acquisition. Re-emergence time is also likely influenced by a tradeoff between predation risk and foraging requirements (Sih 1997, Krause et al. 1998). The longer a prey remains in shelter after having retreated from a predator, the greater likelihood that the predator has gone away. However, time spent hiding in shelter detracts from time spent outside of the shelter foraging. Thus, the longer re-emergence time of *N. longus* may be better suited for predator avoidance and shorter re-emergence time of *C. saepepallens* better suited for foraging. Distance-to-shelter is also likely influenced by predation risk and foraging requirements. By constantly remaining close to shelter, a prey reduces predation risk, but may also reduce its foraging opportunities (Dill 1990). I have found that *N. longus* is a visual sit-and-wait predator and *C. saepepallens* is a roaming winnower (Chapter 3). Thus, foraging mode of the two species is related to distance-to-shelter.

Summary

These results add to the body of evidence that mutualism is more beneficial for obligate mutualists than facultative mutualists and that obligate mutualists have traits which help them derive greater benefit (in this case, avoiding predators). However, these results also suggest that such traits that help obligate mutualists derive greater benefit may impose a cost on other requirements (in this case foraging). This cost may constrain a mutualist species to a less beneficial association with a mutualist partner. Further clarification of the foraging mode, diet, and energetic requirements of the two goby species will provide further evidence that foraging requirements may constrain effective use of shrimp burrows for predator avoidance.

2.5 Literature Cited

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Table 2.1 Results of multiple one-way ANOVAs on different variables related to goby reaction to a model predator. The first five ANOVAs are for no-shelter trials. The last ANOVA is for shrimp-burrow trials. For each ANOVA, the independent variable is goby species (*N. longus* or *C. saepepallens*).

<i>Flight Initiation Distance with No Shelter</i>					
Source	df	SS	MS	F	P-value
Species	1	0.28	0.28	0.015	0.904
Residuals	47	900.52	19.16		
Total	48	900.8			
<i>Average Speed (cm/sec)</i>					
Source	df	SS	MS	F	P-value
Species	1	164	164	0.236	0.63
Residuals	46	31905	694		
Total	47	32069			
<i>Average Speed (body lengths/sec)</i>					
Source	df	SS	MS	F	P-value
Species	1	139.64	193.64	3.058	0.087
Residuals	46	2913.11	63.33		
Total	47	3052.75			
<i>Top Speed (cm/sec)</i>					
Source	df	SS	MS	F	P-value
Species	1	562	562	0.568	0.455
Residuals	45	44546	990		
Total	46	45108			
<i>Top Speed (body lengths/sec)</i>					
Source	df	SS	MS	F	P-value
Species	1	318.8	318.8	3.182	0.081
Residuals	45	4508.5	100.2		

Total	46	4827.3			
<i>Flight Initiation Distance at Burrow Entrance</i>					
Source	df	SS	MS	F	P-value
Species	1	328.3	328.3	4.302	0.053
Residuals	18	1373.53	76.31		
Total	19	1701.83			

Table 2.2 Results from *in situ* observations of gobies on two variables: re-emergence time (duration spent in a burrow after being frightened) and flight initiation distance (distance from a SCUBA diver when a focal goby began its retreat). Presented here are two two-way ANOVAs with the sites and goby species as independent variables.

<i>Re-emergence time</i>					
Source	df	SS	MS	F	P-value
site	1	4211	4211	0.237	0.627
species	1	39882	39882	22.45	< 0.001
site*species	1	1601	1601	0.09	0.765
residuals	101	1794543	17768		
total	104	1840237			
<i>Flight initiation distance</i>					
Source	df	SS	MS	F	P-value
site	1	2493	2493	4.338	0.039
species	1	36023	36023	62.69	< 0.001
site*species	1	1310	1310	2.28	0.1342
residuals	101	58038			
total	104	97864			

Table 2.3 Results of two-way permutation MANOVA with species and site as independent variables. The dependent variables are time spent in different distances from shelter (three bins total, < 10, 10-30, and >30 cm from shelter).

Source	df	SS	MS	F model	P-value
Species	1	0.826	0.826	18.948	0.001
Site	1	0.010	0.010	0.225	0.824
Species*Site	1	0.047	0.047	1.073	0.308
Residuals	76	3.313	0.044		
Total	79	4.195			

Figure 2.1 Orientation of a focal goby in relation to the model predator that moves from right to left. Letters refer to the direction the goby was facing, i.e. “A” toward the model predator, “B” sideways to the predator, and “C” away from the model predator.

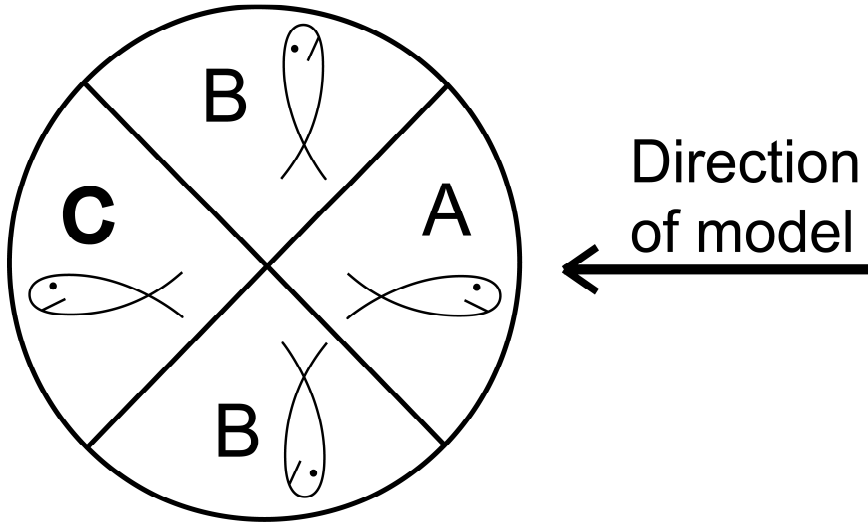


Figure 2.2 Mean survival time for the two goby species using three different shelter types during staged interactions with a live predator in 190-l aquaria. Error bars are 95% confidence intervals. Letters above bars indicate differences using survival analysis with the appropriate Bonferonni correction (see text).

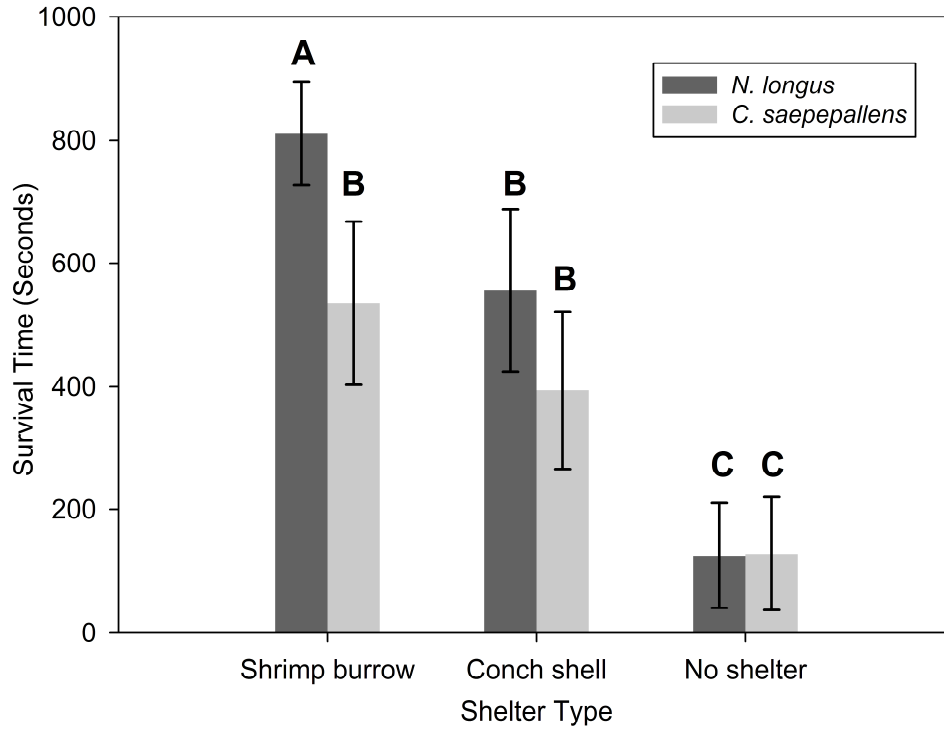


Figure 2.3 Left axis: flight initiation distance for the two goby species and two sites. Right axis: emergence time (after being frightened into a burrow) for the two goby species. Emergence time data are combined from both sites because site was found to have no effect on emergence time (Table 2.2). Error bars are 95% confidence intervals.

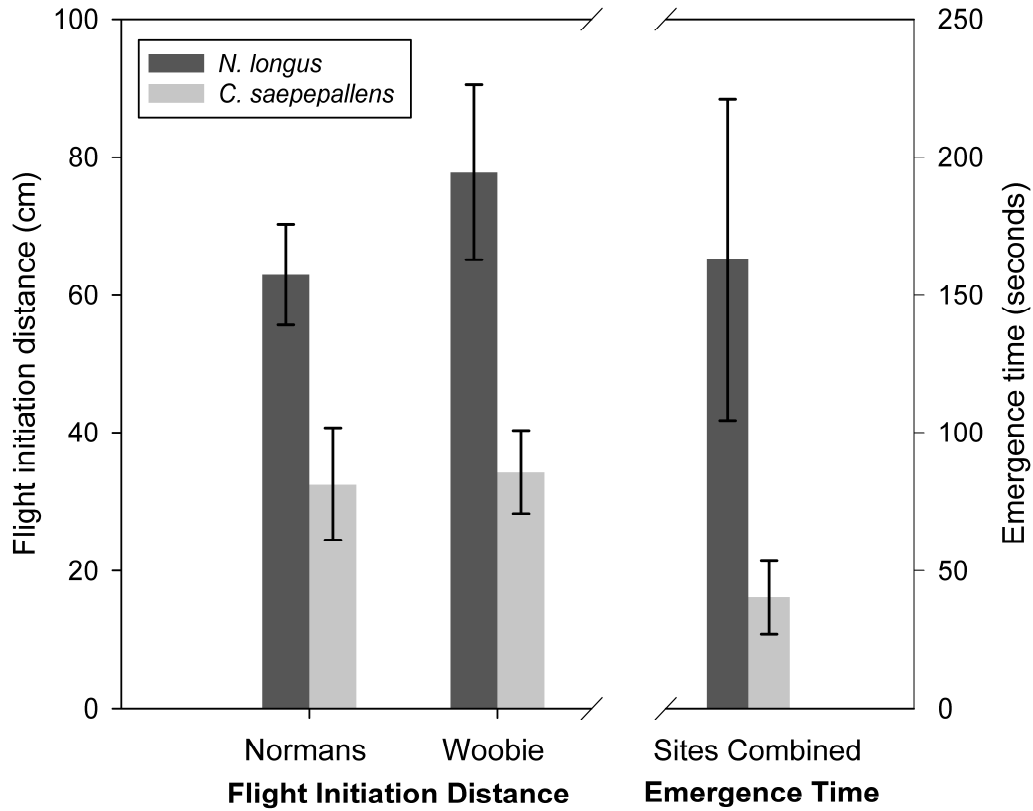
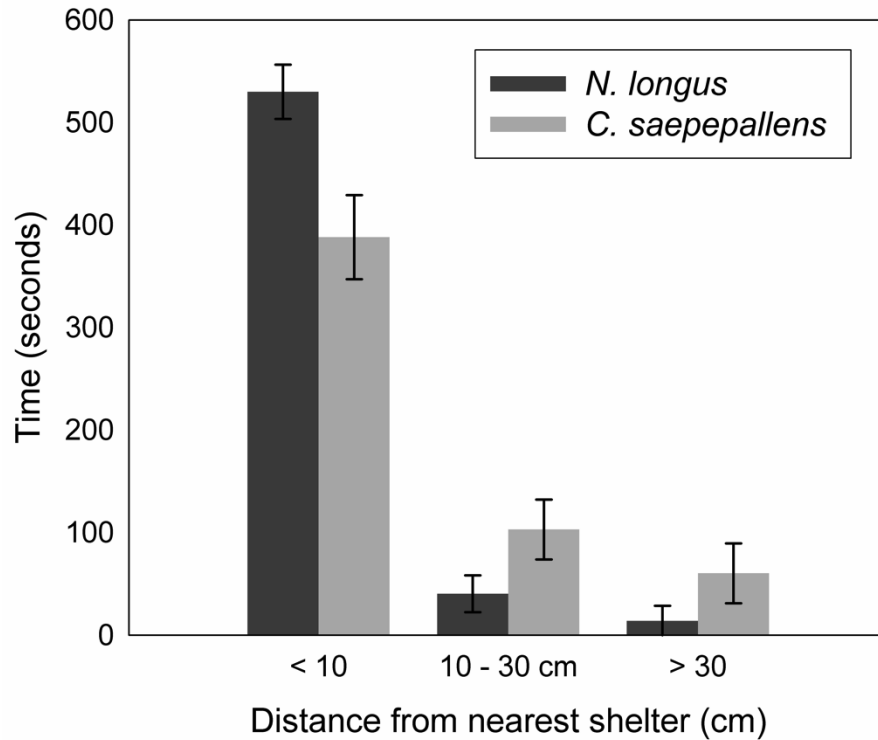


Figure 2.4 Average time spent at different distances-to-shelter. Data are compiled from both sites at which observations were conducted as it was found that site had no effect on distance-to-shelter. Error bars are 95% confidence intervals.



Chapter 3. Foraging requirements as a constraint on a shrimp-goby mutualism

Abstract A mutualism occurs between alpheid shrimp and gobiid fishes in which poor-sighted shrimp share their burrows with individual goby partners. Gobies act as sentinels for shrimp. In the Western Atlantic, an obligate shrimp-associated goby (*Nes longus*) and a facultative shrimp-associated goby (*Ctenogobius saepepallens*) both associate with the shrimp, *Alpheus floridanus*. Mutualism probably imposes a foraging cost to gobies, because gobies communicate with shrimp via physical contact and must, therefore, remain at burrow entrances. However, only *N. longus* maintains constant a position at burrow entrances. In the vicinity of Lee Stocking Island, Bahamas, sediment infauna were less abundant at *A. floridanus* burrow entrances than elsewhere, indication of a potential foraging cost for *N. longus*. However, when placed on a restricted diet, *N. longus* lost less mass and had lower mortality than *C. saepepallens*. Thus, *N. longus* is better equipped for coping with its restricted feeding conditions than *C. saepepallens*. This difference is likely due to differences in activity level and foraging style: *N. longus* is an inactive sit-and-wait predator at *A. floridanus* burrow entrances and *C. saepepallens* is an active winnower that forages over broad areas. Thus, differences in how the two goby species associate with *A. floridanus* may be related to differences in energetic requirements and foraging style. The active winnowing strategy of *C. saepepallens* may constrain it to a casual and facultative association with *A. floridanus*.

3.1 Introduction

Mutualism is an interspecific interaction in which both species receive a net benefit. Receiving a resource from a partner species is beneficial, but providing a resource to a partner species is often costly (Bronstein 2001). For example, figs and yuccas benefit from pollination by fig-wasps and yucca-moths, respectively, but female pollinators in both cases also oviposit eggs into the flowers of their hosts. Upon hatching, pollinator offspring consume a proportion of the seeds, the loss of which is a direct cost to the host plant (Bronstein 2001).

Mutualist species vary in their interdependency. Obligate mutualists rely on a resource provided by their mutualist partner so much that they cannot survive without it (Boucher et al. 1982). In contrast, facultative mutualists benefit from a resource provided by their mutualist partner, but can survive without it because they either can gain that resource through other means or do not need that resource for survival (Boucher et al. 1982).

If the mechanisms by which a species copes with the cost of mutualism (provisioning a resource) are ineffective or non-existent, than a loosely-associated facultative relationship may be more likely to develop than a tightly-associated obligate relationship. The likelihood for evolution of obligate mutualism might be enhanced if the interactions between two species have pre-adaptations that reduce cost (Pellmyr et al. 1996). Alternatively, mechanisms for coping with cost of mutualism could be gained through coevolution (Thompson 1994). Here, I compare the effectiveness by which two species (one facultative and one obligate mutualist) cope with the cost of mutualism.

A mutualism occurs between gobiid fishes and alpheid shrimp in which a shrimp individual maintains a burrow that it shares with an individual goby (Karplus 1987, Karplus &

Thompson 2011). Both the shrimp and goby use these burrows for avoiding predators. Thus, the benefit to the goby is shelter from predators, and gobies without shrimp partners are quickly eaten when predators are present (Thompson 2005). These shrimp have poor vision and are prone to predation while foraging outside the burrow or maintaining the burrow entrance. Gobies remain at burrow entrances, and shrimp use two behaviors of gobies that emergence is unsafe (Preston 1978). The first is a fluttering of the caudal fin on the antennae of the shrimp, and the second, a headfirst retreat into the burrow. Shrimp without gobies rarely emerge from their burrows and suffer a growth decrement (Thompson 2003).

Over 120 goby species and 20 alpheid shrimp species participate in this mutualism. The vast majority of these gobies have been considered obligate mutualists and use both warning behaviors described above (Karplus 1987, Karplus & Thompson 2011). Only four goby species have been described as facultative mutualists, one in Japan (Yanagisawa 1978, 1984) and three in the Western Atlantic (Wayman 1973, Weiler 1976, Karplus 1992, Randall et al. 2005, Kramer et al. 2009a). Facultative shrimp-associates use burrows for predator avoidance, just as obligate shrimp-associates do, but rarely or never use the caudal fin-fluttering signal. In the Western Atlantic, the three facultative gobies, including *Ctenogobius saepepallens*, and one obligate goby, *Nes longus*, all associate with a single shrimp, *Alpheus floridanus*. *C. saepepallens* and *N. longus* are not sister species to each other or to any shrimp-associated gobies (Rüber et al. 2003, Thacker 2003). Previously, I found that *C. saepepallens* spends less time at burrow entrances signaling shrimp, has weaker preference for shrimp burrows versus other shelters, and forms shorter duration associations with shrimp (5.48 minutes for *C. saepepallens* versus 2.52 days for *N. longus*; Chapter 1).

Gobies generally feed on mobile infauna such as small crustaceans. *N. longus* visually locate emerging infauna as sit-and-wait predators and dart short distances when attacking prey. In contrast, *C. saepepallens* feeds over broader areas than *N. longus* by winnowing, i.e. engulfing sand and trapping food items on the gill rakers (Langeland & Nøst 1995, McCormick 1998). Because communication with shrimp is through physical contact (Preston 1978), an obligate mutualism involving caudal fin fluttering may require that goby foraging only take place at the burrow entrance. This may be costly to foraging and thus, one principal cost of mutualism to the goby may be foraging efficiency. This cost may cause depletion of local food abundance at burrow entrances, and place an upper limit on the energetic demands that can be satisfied by foraging while maintaining communication with shrimp. It is likely that *N. longus* has lesser energetic demands than *C. saepepallens*, thus allowing maintenance of associations with *A. floridanus*.

I pose four hypotheses related to the relative confinement of gobies near shrimp burrow entrances, the relative costs involved, and the mechanisms compensating for those costs:

(1) *Reduced benthic infaunal abundance near shrimp burrow entrances.* The abundance of small infaunal invertebrates, such as copepods, will be less at burrow entrances than elsewhere due to the heightened foraging effort of *N. longus*, and to a lesser extent *C. saepepallens*, at burrow entrances.

(2) *Resistance to starvation.* When placed on a restricted diet, *N. longus* will lose less mass and have lower mortality than *C. saepepallens*.

(3) *Energetic cost of movement*. While in small containers, similar in size to the entrances of shrimp burrows, *N. longus* will expend less energy by adjusting its position less often than *C. saepepallens*.

(4) *Diet specialization*. The two species will consume similar infaunal prey items but in different proportions. Because *C. saepepallens* feeds by winnowing, its diet will match local abundance of infaunal invertebrates more closely than will the diet of *N. longus*.

3.2 Methods

Study sites

All experiments and sample collections took place at the Perry Institute for Marine Sciences, Lee Stocking Island, Exuma, Bahamas during the 2011 summer. I used two field sites: Normans (23°45'35.64"N, 76° 7'59.64"W), which is composed of bare sand with coral rubble and various seagrasses and Woobie (23°49'4.55"N, 76°11'17.43"W), which is entirely bare sand with shrimp burrows offering the only shelter. At the more complex site Normans, two other goby species are abundant: *Coryphopterus glaucofraenum* and *Gnatholepis thompsoni*. At site Woobie, the goby *Oxyurichthys stigmaliophius* is also present but rare. A quantitative description of these two sites is in the first chapter.

Depletion of sediment infauna at burrow entrances

I collected sediment samples at and away from *A. floridanus* burrow entrances, and quantified abundance of all invertebrates present. I collected sediment cores in a paired fashion: one 5 cm in front of a burrow entrance, and one 50 cm from that burrow entrance (and at least 50 cm from any other burrow), referred to as “open benthos”. An *N. longus* individual was present at each burrow entrance sampled and its size was estimated. I collected 40 pairs of samples from site Normans and 44 pairs from site Woobie (168 samples total).

Sediment cores were collected using a 60-ml syringe barrel. Circular cores measured 2.7 cm in diameter and were taken to a depth of 2.5 cm below the sediment-water interface. Samples were stained and preserved in a solution of Rose Bengal and 5% formalin for 3 days. Samples were then rinsed on a 30- μ m plastic screen and stored in 70% ethanol. I removed all of the invertebrates from the samples using a dissecting scope and then tallied invertebrates using a compound microscope. I placed invertebrates into four taxon groups. (1) “Copepods,” included mostly harpacticoid copepods of the families Ameiridae, Cletodidae, and Harpacticidae, but several other harpacticoid families and few cyclopoids. Copepods were distinguished by the presence of long left/right oriented first antennae and long caudal rami. (2) “Other crustaceans” included the classes Cephalocarida and Ostracoda and orders Cumacea, Tanaidacea, and Gammaridea (Amphipoda). Identification of Cephalocarida was based on a lack of eyes and long abdomen devoid of appendages save two long caudal rami, Ostracoda based on their valves, Cumacea based on their large carapace and relatively thin abdomen segments. Tanaidacea and Gammaridea are somewhat similar but the former has a carapace, 5 pleopods versus 3, and is typically more elongate. (3) “Molluscs” included the orders Bivalvia and Gastropoda. (4) “Nematodes.”

I additionally found both free-living and tube-forming groups of polychaetes and free-living oligochaetes. However, these groups were nearly absent from the digestive tracts of both species (see below). The cause of their absence from digestive tracts may have been rapid decay of their soft tissues and/or because they were not eaten by gobies. Thus, I excluded annelids from analyses of sediment infauna, because I could not determine whether gobies consume them. Several other groups were either in very low abundance in the infauna and/or absent from goby digestive tract contents, so I thus excluded Foraminifera, Ophiuroidea (brittle stars), Sipuncula, and Amphioxiformes (lancelets).

For multivariate analyses of these data, I used *taxon abundance* of each of the four invertebrate taxa as the dependent variables and three independent variables: *site* (Normans or Woobie), *location* (burrow entrance or open benthos), and *goby size* (estimated size of the goby at the burrow entrance: > 4 cm or < 4 cm). Because core samples were collected in a paired fashion, the independent variable *location* was in a paired structure. The variable *goby size* only applied to samples collected at burrow entrances because open benthos sediment cores did not have an associated *N. longus* individual.

Because of the structure of the three independent variables, I used three different analyses to test for their effect on *taxon abundance*, which did not conform to multivariate normality (Shapiro-Wilks test). First, to examine the effect of the independent variable *site* on the multivariate dependent variable *taxon abundance*, I used a permutation one-way MANOVA (Anderson 2001) with the “adonis” subroutine in R (“vegan” package). This test showed that the two sites were different ($F = 57.9$, $df = 1$, $P < 0.001$). Thus, I used two Hotelling’s T² (multivariate analogue of the t-test) to analyze the effect of the independent variable *location* on *taxon abundance* at each site separately (“HotellingsT2” subroutine in R, “ICSNP” package).

Because the data was not normally distributed, the test statistic was based on a chi-square approximation, rather than a F-distribution. Third, I used a one-way permutation MANOVA to analyze the effect of the independent variable *goby size* on *taxon abundance*.

In addition, I examined the effect of the three independent variables on total invertebrate abundance (all four prey taxa summed, *total infauna*). I used a square-root transformation to normalize the data. First, I used a one-way ANOVA with *total infauna* as the dependent variable and *site* as the independent variable. Second, I used two paired t-tests (one for each site, because the two sites were found to be different: $F = 58.5$, $df = 1$, $P < 0.001$) with *total infauna* as the dependent variable and *location* as the independent variable. Third, I used a one-way ANOVA with *total infauna* as the dependent variable, and *goby size* as the independent variable.

Goby diet

Gobies were captured with aquarium nets at site Normans on 7/1/2011 and 7/21/2011 and at site Woobie on 7/2/2011 and 7/23/2011. On each collection day, 25 *N. longus* and 25 *C. saepepallens* were captured (200 total). Gobies were transported to the lab alive and euthanized with an overdose of MS-222. The entire digestive tract of each goby was removed within 5 hours of capture. Digestive tracts were preserved in a 5% formalin solution for 7 days and then stored in 70% ethanol.

When possible, I identified all gut content items to the same level as with the sediment infauna survey (see above). I categorized prey items by the same four invertebrate taxon groups as described above.

First, I examined the effect of three independent variables: *goby species* (*N. longus* or *C. saepepallens*), *site* (Normans or Woobie), and *goby size* (five 10-mm size bins from 20 to 70 mm) on total invertebrate counts in gut contents. Because the data was not normally distributed and log, square root, and reciprocal transformations did not normalize the data, I used a three-way permutation ANOVA (R subroutine “adonis” in “vegan” package).

Second, for each site, I ran a one-way permutation MANOVA with *taxon relative abundance* as the multivariate dependent variable and *origination* (*N. longus* digestive tract, *C. saepepallens* digestive tract, or sediment) as the independent variable. I also ran multiple comparisons using the same analysis to examine differences among *taxon relative abundance* in sediment and in the digestive tracts of both gobies. For each site, I additionally used Principal Components Analysis to visualize the data and examine the contribution of each invertebrate taxon group to variation between the two gobies and sediment (R subroutine “prcomp” in “vegan” package).

Food-restriction assay

I placed gobies of the two species under a restricted diet to compare rate of mass loss of the two species. Gobies between 25 and 45 mm total length were collected at site Normans (39 *N. longus* and 39 *C. saepepallens*). Before the food-restriction assay began, all gobies were blotted to remove excess water, weighed (wet mass), and measured (total length). Starting length was similar between the two goby species (mean \pm 95% confidence: *N. longus*, 35.9 \pm 1.50 mm, *C. saepepallens*, 36.2 \pm 2.00 mm; Kruskal-Wallis $X^2 = 0.425$, $df=1$, $P = 0.514$), although, starting wet mass was different between the two goby species (mean \pm 95% confidence: *N. longus*, 0.242

± 0.027 grams and *C. saepepallens*, 0.319 ± 0.042 grams; Kruskal-Wallis $X^2 = 6.734$, $df=1$, $P = 0.009$).

During the 25-day food-restriction period, gobies were placed individually in clear containers measuring 11 X 11 X 11 cm. A 6-cm long section of 1.25-cm PVC pipe was placed in each container to provide shelter for gobies. Containers were stored in three 190-litre flow-through seawater aquaria (28 containers in each). The location of goby species was randomly assigned among aquaria to control for any difference in water flow. For each aquarium, a 2.5-cm pipe delivered raw seawater into 28 smaller tubes (4-mm inner diameter) into individual containers. Water flowed into containers at a rate between 600 and 1100 ml/minute.

Brine shrimp, *Artemia salina*, were hatched every other day and readily consumed by both goby species. All hatched *Artemia* were distributed evenly among the gobies. The average daily ration of *Artemia* was 0.032 g (wet mass) per goby. Given this ration and the range of size of gobies used, daily diet ranged between 5.5 and 31.0 % of a goby's starting wet mass.

By the end of the 25-day food-restriction period, there was some mortality (2 of 39 *N. longus* and 9 of 39 *C. saepepallens*). I used a goodness-of-fit test to compare mortality between goby species. Remaining gobies were euthanized with an overdose of MS-222, blotted with a paper towel, weighed, and measured.

I calculated *percent mass loss* for each goby as a dependent variable. To examine whether mass loss was different between the two goby species, I conducted an ANOVA with species (*N. longus* or *C. saepepallens*) as the independent variable, aquarium (one of three) as a random effect, and *percent mass loss* as the dependent variable. There were also three continuous independent variables: *average activity* (see below), *daily diet*, and *start mass*. To examine their

effect, I used a three-way ANCOVA with *percent mass loss* as the dependent variable, *goby species* as the categorical independent variable, and three continuous independent variables as covariates (Conover & Iman 1982). I also, tested the effect of *percent mass loss* on *activity* with two linear regressions (one for each species).

Before (6/22/2011) and after (7/17/2011) the food-restriction period, gobies were filmed in the same 11 X 11 X 11-cm containers in which they were held during the food-restriction period. Gobies were filmed from above during 10-minute intervals using either a Casio Exilim FH-100 camera or Canon G11 camera. From these videos, I calculated number of moves/minute, referred to as the dependent variable *activity*. A move is defined as any time a focal goby at rest made any movement and then came to rest again. Moves included both minor movements such as adjustment of position or larger movements such as swimming from one end of the container to the other. *Activity* was normalized using a square-root transformation. I used a repeated-measures ANOVA to test for differences in activity before and after the food-restriction treatment, and between the two species. Because the two species had statistically different activity levels and because there was an interaction between the independent variables *species* and *time*, I used a separate paired t-test for each species to test for a difference in activity before and after the treatment.

3.3 Results

Depletion of sediment infauna at burrow entrances

The location of sediment samples (burrow entrance or open benthos) had a significant effect on infaunal abundance at site Woobie (*Hotelling's T*₂ = 18.33, df = 4, *P* = 0.001; Fig. 3.1)

but not at site Normans (*Hotelling's T2* = 2.445, *df* = 4, *P* = 0.654; Fig. 3.1). At site Woobie, copepods were 67.9%, other crustaceans were 111%, molluscs were 68.4%, and nematodes were 14.1% more abundant in open benthos than at burrow entrances (Fig. 3.1). Site (Normans or Woobie) also had an effect on infaunal abundance (*F* = 57.9, *df* = 1, *P* < 0.001; Fig. 3.1). Copepods were 25.7% less abundant, other crustaceans were 310% more abundant, molluscs were 0.7 % less abundant, and nematodes were 297% less abundant at site Normans than Woobie (Fig. 3.1). Goby size (< 4 cm or > 4 cm) had no effect on sediment infaunal abundance (*F* = 0.616, *df* = 1, *P* = 0.638).

Total infaunal abundance was greater in open benthos than burrow entrances at site Woobie (t-test: *t* = 2.23, *df* = 43, *P* = 0.031; Fig. 3.1), but not at site Normans (t-test: *t* = 0.002, *df* = 39, *P* = 0.99; Fig. 3.1). Total infaunal abundance was greater at site Woobie than site Normans (ANOVA: *F* = 58.5, *df* = 1, *P* < 0.001; Fig. 3.1). Goby size had no effect on total infaunal abundance (ANOVA: *F* = 0.69, *df* = 1, *P* = 0.41).

Goby diet

Total invertebrate abundance was greater in the in digestive tracts of *C. saepepallens* than of *N. longus* (Fig. 3.2, Table 3.1). The digestive tracts of *C. saepepallens* at site Woobie had statistically greater total invertebrate abundance than at site Normans (Fig. 3.2, Table 3.1). Goby size (five 10-mm size bins from 20 to 70 mm) had no effect on total invertebrate abundance in digestive tracts.

At site Normans, the relative abundance of the four invertebrate taxa in the sediment was different from the digestive tracts of *N. longus* (*F* = 53.02, *df* = 1, *P* < 0.001) and *C. saepepallens*

($F = 9.01$, $df = 1$, $P < 0.001$; Fig. 3.3A). *N. longus* digestive tracts contained proportionally less copepods, more other crustaceans, more molluscs, and less nematodes than sediment samples (Fig. 3.3A). *C. saepepallens* digestive tracts contain proportionally more copepods than sediment samples and similar other crustaceans, molluscs, and nematodes as sediment samples (Fig. 3.3A). Relative invertebrate taxa abundance was statistically different between the digestive tracts of the two goby species ($F = 41.91$, $df = 1$, $P < 0.001$). *N. longus* digestive tracts contained proportionally less copepods, more other crustaceans, more molluscs, and less nematodes than *C. saepepallens* (Fig. 3.3A). The diet of *C. saepepallens* was more closely matched than the diet of *N. longus* with sediment infauna, at least on the first principal components axis, which accounted for 42.4% of the variation and was most strongly correlated with copepods and other crustaceans (loadings = -0.57 and 0.69, respectively; Fig. 3.3B).

At site Woobie, the relative abundance of the four invertebrate taxa in the sediment was statistically different from the relative abundance in the digestive tracts of *N. longus* ($F = 77.74$, $df = 1$, $P < 0.001$) and *C. saepepallens* ($F = 5.96$, $df = 1$, $P = 0.003$, respectively; Fig. 3.3C). *N. longus* digestive tracts contained proportionally less copepods and nematodes, and more other crustaceans and molluscs than sediment samples (Fig. 3.3C). *C. saepepallens* digestive tracts and sediment samples contained similar proportions of copepods, other crustaceans, molluscs, and nematodes (Fig. 3.3C). Relative invertebrate taxa abundance was statistically different between the digestive tracts of the two goby species ($F = 39.20$, $df = 1$, $P < 0.001$). *N. longus* digestive tracts contained similar proportions of copepods as *C. saepepallens* digestive tracts, but more other crustaceans and molluscs and less nematodes (Fig. 3.3C). The diet of *C. saepepallens* was more closely matched than the diet of *N. longus* with sediment infauna on the first principal components axis, which account for 44.4% of the variation and was most strongly correlated

with other crustaceans, molluscs, and nematodes (loadings = -0.49, -0.51, and 0.67, respectively), and the second principal components axis, which accounted for 29.3% of the variation and was most strongly correlated with copepods and molluscs (loadings = -0.71 and 0.49, respectively; Fig. 3.3D).

Food-restriction assay

Mortality was higher in *C. saepepallens* than in *N. longus* (9/39 versus 2/39; goodness-of-fit: $X^2 = 3.81$, $df = 1$, $P = 0.051$). *C. saepepallens* also lost more mass than did *N. longus* (mean \pm 95% confidence: 47.1 ± 2.49 versus 34.4 ± 2.98 percent body mass, respectively; ANOVA: $F = 40.24$, $df = 1$, $P < 0.001$). There was no interaction between species and aquarium (ANOVA: $F = 2.60$, $df = 2$, $P = 0.082$).

Average activity and starting mass had no effect on percent mass loss in gobies (Table 3.2). Average daily diet did have an effect on mass loss: greater percent daily diet was associated with lower percent mass loss (Table 3.2). Mass loss had no effect on post-treatment activity for *N. longus* (Linear Regression: $t = -1.54$, $n = 29$, $P = 0.134$) or *C. saepepallens* (Linear Regression: $t = -0.308$, $n = 25$, $P = 0.760$).

C. saepepallens was more active than *N. longus* (mean \pm 95% confidence: 9.86 ± 1.20 moves/min versus 4.77 ± 0.92 , respectively; ANOVA: $F = 39.7$, $df = 1$, $P < 0.001$). There was no difference in activity before and after the food-restriction treatment (ANOVA: $F = 0.263$, $df = 1$, $P = 0.61$), but there was an interaction between species and time (ANOVA: $F = 5.60$, $df = 1$, $P = 0.022$). *C. saepepallens* was less active before than after the food-restriction treatment (8.97 ± 1.66 versus 11.6 ± 1.57 moves/min, respectively; t-test: $t = 2.49$, $df = 23$, $P = 0.020$). *N. longus*

had similar activity before and after the food-restriction treatment (5.35 ± 1.11 versus 4.81 ± 1.60 moves/min, respectively; t-test: $t = 1.11$, $df = 26$, $P = 0.28$).

3.4 Discussion

The cost of mutualism

Because obligate mutualists are more reliant on their partners than facultative mutualists, significantly greater costs might be expected in the process of maintaining the obligate association. I previously found that *N. longus* benefits more than *C. saepepallens* from association with shrimp. *N. longus* avoided predators more effectively while using shrimp burrows than shells as refuge. In contrast, *C. saepepallens* avoided predators with equal effectiveness while using shrimp burrows and shells (Chapter 2). Thus, for *N. longus*, there is greater payoff for maintenance of associations with *A. floridanus*, i.e. maintaining position at *A. floridanus* burrow entrances. It is not surprising then that *N. longus* remain with *A. floridanus* for longer periods and have greater preference for *A. floridanus* burrows rather than other shelters than *C. saepepallens* (Chapter 1).

Here, I provide evidence that depending on the energetic needs of a goby, there may be a cost associated with confining foraging effort to burrow entrances. At one site (Woobie), food supply at burrow entrances was less abundant than elsewhere. This depletion likely resulted from the focused foraging of *N. longus*, and to a lesser extent *C. saepepallens*, at shrimp burrow entrances.

Importantly, this pattern was not present at a second site (Normans), indicating that this cost varies spatially. The lack of apparent food depletion at burrow entrances at site Normans was not due to differences of foraging effort of *N. longus* and *C. saepepallens* between site Normans and Woobie. I previously found that at site Normans, *N. longus* and *C. saepepallens* were at burrow entrances 87.5% and 42.6% of the time, respectively. At site Woobie, *N. longus* and *C. saepepallens* were at burrow entrances 89.1% and 45.3% of the time, respectively (Chapter 1). However, two other abundant goby species (*Corphopterus glaucofraenum* and *Gnatholepis thompsoni*) that were found at site Normans, but not at site Woobie, may deplete infaunal invertebrates in open benthos. Both species forage on each of the invertebrate taxa studied here (Wayman 1973, Kramer et al. 2009b). *C. glaucofraenum* was present at shrimp burrow entrances only 20.5% of the time, whereas *G. thompsoni* used coral rubble and patch coral for shelter and was not observed using shrimp burrows (Chapter 1). Thus, depletion of infaunal invertebrates by *N. longus* and *C. saepepallens* at burrow entrances may be matched by depletion of infaunal invertebrates in open bottoms distant from shrimp burrows by *C. saepepallens*, *C. glaucofraenum*, and *G. thompsoni*. In addition, nocturnal emergence rates may differ between the Normans and Woobie sites (Alldredge & King 1977). Greater emergence rates at Normans could act to homogenize meiofauna abundance between burrow entrances and elsewhere.

Relation of degree of mutualistic association by gobies to energetic consequences

While foraging on a limited diet in a confined area, similar in size to the entrance of a shrimp burrow, *N. longus* suffered lower mortality and lost less body mass than *C. saepepallens*.

Thus, *N. longus* is better equipped for coping with the cost of remaining at food-deplete burrow entrances than *C. saepepallens*. The difference in performance on a restricted diet was likely related to differences in activity between the two species. *N. longus* moved less frequently while in a confined area *in vivo* (see above) and between burrows *in situ* than *C. saepepallens* (Chapter 1). By moving less, *N. longus* spends more time in a resting metabolic state and likely has less energetic needs than *C. saepepallens* (see Huey & Pianka 1981).

Activity level is related to foraging style, which differed greatly between the two goby species. *N. longus* is a sedentary visual sit-and-wait predator that targets crustaceans, particularly tanaidaceans and cumaceans, as well as gastropod and bivalve molluscs. *N. longus* likely lacks nematodes from its diet because nematodes may be below the lower size limit for visual detection (Hairston et al. 1982, Li et al. 1985) or because nematodes rarely emerge from the sediment at night or day (Ohlhorst 1982, Youngbluth 1982, Walters & Bell 1986, Walters 1988). Adult gastropods and bivalves do not emerge either (Walters & Bell 1986), but many are found near the water-sediment interface and may become exposed to visual predators such as *N. longus*. In contrast, *C. saepepallens* is a roaming winnower that consumes all benthic meiofauna (including nematodes) in roughly the proportion in which they occur in the benthos. The greater abundance of food items in the digestive tracts of *C. saepepallens* than *N. longus* is probably caused by the lower diet selectivity of *C. saepepallens* compared to *N. longus*. Depczynski & Bellwood (2004) compared time budgets of seven small coral reef fishes (six gobies and one blenny) including two roaming winnowers and five sit-and-wait predators. Winnowers spent more time moving and feeding than sit-and-wait predators. The winnowers had similar diets that were more specialized than two of the sit-and-wait predators but broader than two other sit-and-wait predators (Depczynski & Bellwood 2003). Thus, while sit-and-wait predatory fishes

certainly move less than winnowing fishes, they do not always have more selective diets than winnowing fishes.

There are two reasonable, but contrasting, hypotheses one could make regarding the relationship between diet breadth and foraging range (i.e. sit-and-wait versus roaming). First, one could argue that, by remaining in one place, an animal is likely to have less foraging opportunity and should therefore incorporate a wider diet than a roaming predator. For example, of three crabs differing in mobility, the most mobile had the most specialized diet and the least mobile had the widest diet (Stachowicz & Hay 1999). Alternatively, one could argue that a sit-and-wait predator does not use as much energy as a roaming predator and can therefore subsist on a more specialized diet. For example, pollock (*Pollachius virens*) that are sit-and-wait predators and saithe (*Pollachius pollachius*) that are roaming predators occur in the same kelp beds, but pollock have a narrower diet breadth (Sarno et al. 1994). There is a problem of causality here. Mobility and a high energetic demand may force the adoption of a broad diet. Alternatively, a specialized diet may force the adoption of a large foraging range.

For *N. longus* and *C. saepepallens*, it is unclear whether diet breadth or foraging style is the causal mechanism. However, it is clear that for communication with shrimp, a sit-and-wait feeding strategy is probably most suitable and may in fact be necessary. Communication with shrimp requires physical contact between the antennae of the shrimp and caudal fin of the goby (Preston 1978). A goby waiting for prey to emerge from the sediment can do so while also acting as a sentinel for shrimp. Thus, it seems reasonable that the foraging strategy of *N. longus* makes it better suited than *C. saepepallens* for a tight association with *A. floridanus*. Because *C. saepepallens* is a winnower with greater energetic demands, or perhaps a need for a broader diet, it must roam and is therefore likely limited to a casual facultative association with *A. floridanus*.

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Table 3.1 Results of a three-way permutation ANOVA with total invertebrate abundance in digestive tract contents of gobies as the dependent variable and three independent variables: Species (*N. longus* or *C. saepepallens*), Size (five 10-mm size bins from 20 to 70 mm) and Site (Normans or Woobie).

Source	Df	SS	MS	F model	P-value
Species	1	4.014	4.014	44.14	< 0.001
Size	4	0.653	0.163	1.795	0.093
Site	1	0.853	0.853	9.382	< 0.001
Species * Size	3	0.273	0.091	1.002	0.416
Species * Site	1	0.599	0.599	6.591	0.003
Size * Site	4	0.313	0.078	0.861	0.552
Species *Size *Site	2	0.053	0.027	0.295	0.886
Residuals	163	14.83	0.091	0.687	
Total	179	21.58	1		

Table 3.2 ANCOVA with *percent mass loss* as the dependent variable, *goby species* as the categorical independent variable, and three different covariates: *activity* (moves/minute), *daily diet* (percent start mass), *start mass* (g)

Source	Estimate	Std. Error	t	P-value
Intercept	0.735	0.142	5.186	< 0.001
Species	-0.148	0.027	-5.353	< 0.001
Start mass	-0.366	0.251	-1.444	0.1539
Activity	-0.003	0.003	-0.972	0.3351
Daily diet	-2.030	1.022	-1.987	0.0516

Figure 3.1 Mean abundance of sediment infauna from each of four invertebrate taxa as well as total abundance of all taxa combined. For both Normans entrance and open benthos, n = 40. For both Woobie entrance and open benthos, n = 44. Error bars are 95% confidence intervals.

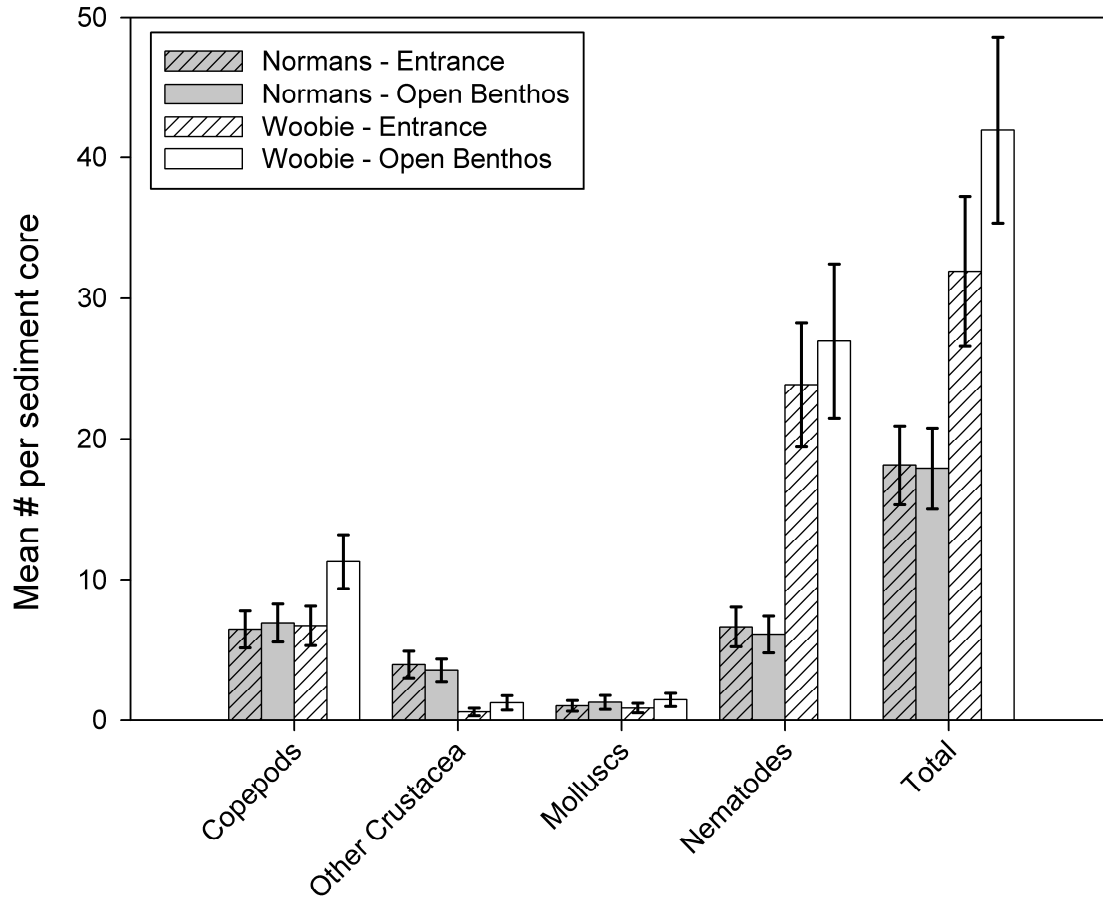


Figure 3.2 Total gut contents of both goby species at both sites. For each bar, n = 50. Error bars are 95% confidence intervals.

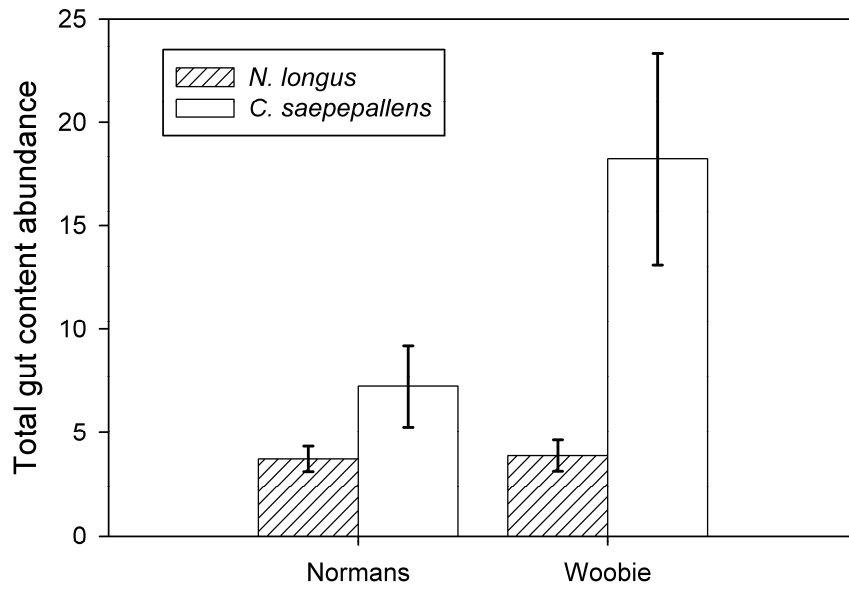
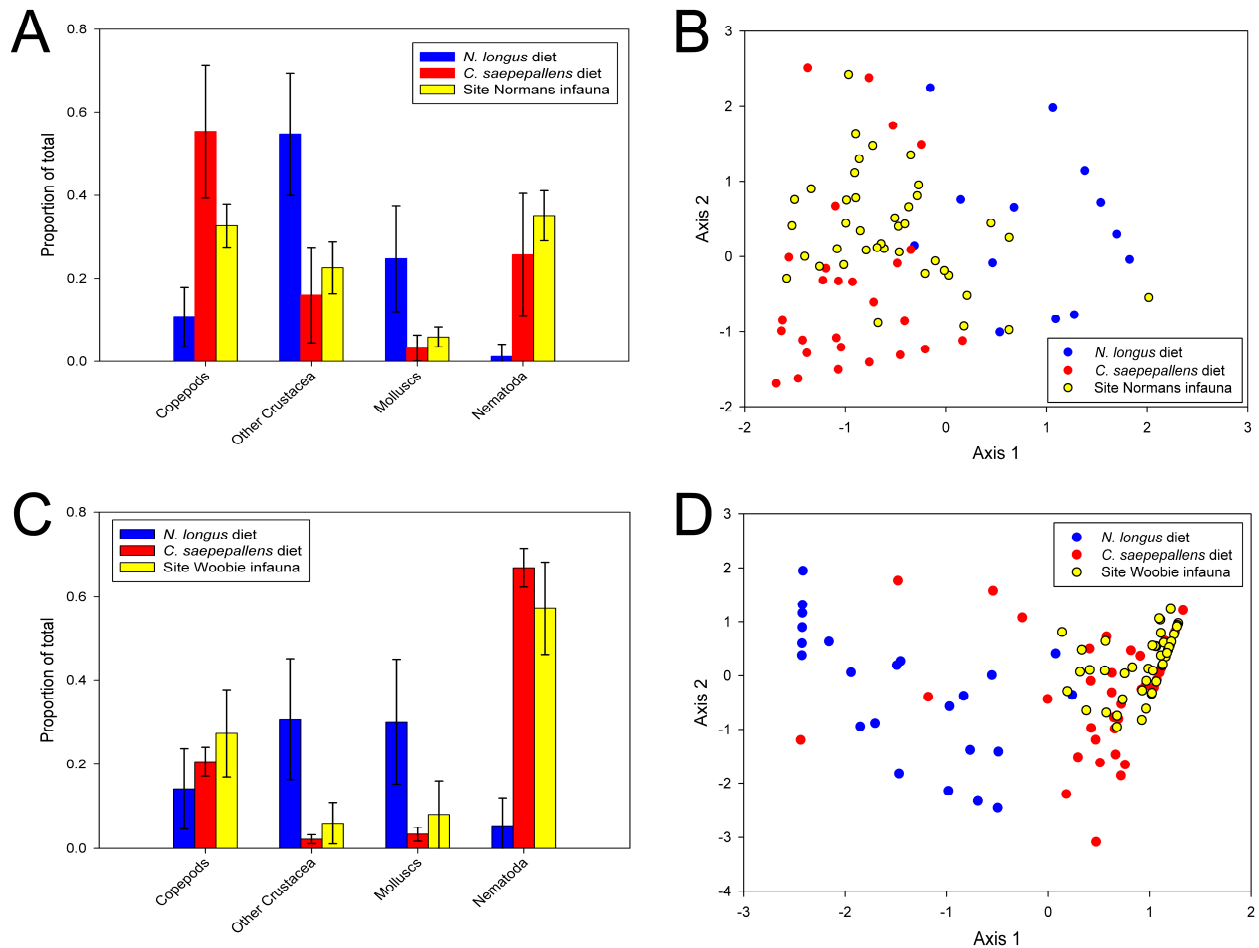


Figure 3.3 Comparisons of digestive tract content of gobies with sediment infauna from shrimp burrow entrances using barplots and Principal Components Analysis (PCA). (A) Mean proportion of invertebrate abundance by taxon found in digestive tracts of each goby species and in sediment infauna from shrimp burrow entrances, all from site Normans. Error bars are 95% confidence intervals. (B) PCA with dependent variables as relative abundance of each of the invertebrate taxa in 3A. The gobies are from site Normans as well as the sediment infauna. Axis 1 and 2 account for 42.4 and 29.6% of the variation, respectively. Axis 1 is most strongly correlated with “copepods” and “other crustaceans” (loadings = -0.57 and 0.69, respectively). Axis 2 is most strongly correlated with “copepods” and “nematodes” (loadings = -0.59 and 0.65). (C) Same as A, but the gobies and sediment infauna are from site Woobie. (D) Same as B, but the gobies and sediment infauna are from site Woobie. Axis 1 and 2 account for 44.4 and 29.3% of the variation, respectively. Axis 1 is most strongly correlated with “other crustaceans,” “molluscs,” and “nematodes” (loadings = -0.49, -0.51, and 0.67, respectively). Axis 2 is most strongly correlated with “copepods” and “molluscs” (loadings = -0.71 and 0.49, respectively).



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