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Host-parasite Interactions between a Copepod (*Pharodes tortugensis*) and Small Reef-associated Gobies (*Coryphopterus*) in the British Virgin Islands

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The effects of parasitic copepods on free-living hosts are infrequently documented, and the copepod *Pharodes tortugensis* has remained virtually unstudied since described. For the first time, we document its host range in the British Virgin Islands (BVI), the prevalence and intensity of infections on wild hosts, and its impacts on host morphology and performance. Infections were observed on four benthic gobies in the BVI (*Coryphopterus glaucofraenum*, *C. venezuelae*, *C. dicrus* and *C. eidolon*) but not on other host species previously reported from other parts of the western Atlantic. Infected gobies were widespread in the BVI (detected at 33 of 52 sites, prevalence from 1–25%) but extremely rare elsewhere in the Caribbean (detected at 2 of 16 sites, prevalence < 0.006%). As is typical of macroparasite infections, *P. tortugensis* was over-dispersed in BVI host populations (mean intensity = 4.7, range = 1–17). Infections were most common in juvenile and female hosts, and rarely found in larger male hosts. The copepods attach in the branchial chamber of the goby; female copepods show high attachment fidelity to the ventral surface of the chamber, while male copepods attached most often to the first two gill arches and in the branchial chamber adjacent to the female. Infections caused substantial damage to the host's branchial chamber and gill filaments. Parasitized gobies also had larger livers and smaller gonads than unparasitized individuals of similar length. The changes in organ mass of infected gobies were not sizeable enough to affect total body mass, and host condition (the body-length vs. body-mass relationship) was similar for gobies with and without infections. Parasitized gobies were, however, significantly smaller in body mass at a given age, reflecting slower overall growth. Effects of *P. tortugensis* on individual hosts were broadly similar to those of other parasitic copepods that infect fish gills and, for unknown reasons, the BVI appears to be a persistent hotspot of infections on these goby hosts.

Key words: Coral reef fish, Ectoparasite, Gill pathology, Host-range, Infection intensity, Liver condition, Prevalence, Reproductive output.

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BACKGROUND

The effects of parasites on fish are generally better documented for microparasites (viruses, bacteria and protozoa) than macroparasites (helminthes and arthropods including cymothoid isopods), whose impacts are thought to be chiefly sublethal (Sindermann 1987; Sale 2002). Copepods are the most common and diverse macroparasites of marine fish (Boxshall and Hayes 2019), and most of what is known about the effects of parasitic copepods on fish hosts is derived from studies of commercial aquaculture. This bias is understandable because parasitic infections in this setting result in potentially severe financial losses (Johnson et al. 2004 2019). Parasitic copepods commonly occupy the gill cavity, oral cavity or skin of their hosts, and their attachment often causes structural damage to the tissues in the area (Kabata 1984). Other pathological consequences of attachment in aquaculture settings include damage to the tongue and sensory organs, whereas parasite feeding can also cause damage to musculature and sometimes atrophy of internal organs (Kabata 1984; Johnson et al. 2019; Kottarathil et al. 2019; Aneesh and Kappalli 2020). Coincident stress-related physiological responses, including anaemia, and altered immune function are often observed (Kabata 1984). At the individual level, infection can reduce the energy available for growth and reproduction so that hosts condition (body mass at a given length) and reproductive output is diminished (Johnson et al. 2019). These impacts on individuals can be severe enough to reduce survival, either directly or indirectly by making infected individuals more vulnerable to other agents of mortality, which can translate to population-level impacts. Aquaculture settings, however, differ from natural ones is ways that may alter host-parasite dynamics (*e.g.*, crowding, stress and diet) and magnify the impact on hosts (Johnson et al. 2004). Further study of parasitic copepods under natural conditions is thus important to understand whether they have equivalent impacts on wild hosts (Johnson et al. 2019; Sikkel and Welicky 2019; Timi and Poulin 2020).

We describe effects on their free-living hosts of a parasitic copepod *Pharodes tortugensis* (Wilson), a member of the family Chondracanthidae found in the western Atlantic (Milne Edwards) (Ho 1970; Østergaard et al. 2003; Hadfield 2019). Chondracanthids are all highly modified

parasites of marine fishes, and relatively little is known of the 193 species in the family aside from morphological descriptions and phylogenetic analysis (Smit et al. 2019). Most Chondracanthids are sexually dimorphic, with dwarf males that are attached to the female. Male *Pharodes* are distinctive because they attach to the fish host independently from the females and males are larger relative to the size of the female than most other species (Ho 1971a; Østergaard and Boxshall 2004). Evidence for the impact of chondracanthids on their hosts is limited to a few case studies. For example, *Chondracanthus goldsmidi* (Tang, Andrews and Cobcroft) attaches to the gills, inner operculum and nasal cavities of its hosts (Andrews et al. 2010). Hosts suffer structural damage at the attachment site that includes including swelling and tissue necrosis (Andrews et al. 2010), with associated overexpression of inflammatory cytokines (Covello et al. 2009). *Pharodes banyulensis* (Delamare Deboutteville and Nunes-Ruivo), a close relative of *P. tortugensis* found in the Mediterranean, also causes substantial physical damages to the gill cavity that compromises the respiratory function of a common host, the Mediterranean blenny *Salaria pavo* (Risso) (Rousset and Raibaut 1984). *P. tortugensis* and *P. banyulensis* are morphologically very similar, differing only slightly in the morphology of the tip of the caudal process of the female copepods; and these variations could be due to intraspecific variation among localities (Ho 1971a).

We discovered *P. tortugensis* infecting four species of goby that inhabit mixed reef and coral reef habitats. Three common gobies, *Coryphopterus glaucofraenum* (Gill), *C. venezuelae* (Cervigón) and *C. dicrus* (Böhlke & Robins) are infected, as is a much rarer goby, *C. eidolon* (Böhlke & Robins). Infected gobies were first noticed in 1993 because their physical appearance differs from that of uninfected gobies: copepods attached in the host branchial chamber cause visible distension of the operculum (Fig. 1). Using this visual criterion, divers can accurately identify unparasitized (92% accuracy) and parasitized (84% accuracy) gobies during underwater surveys (Finley and Forrester 2003).

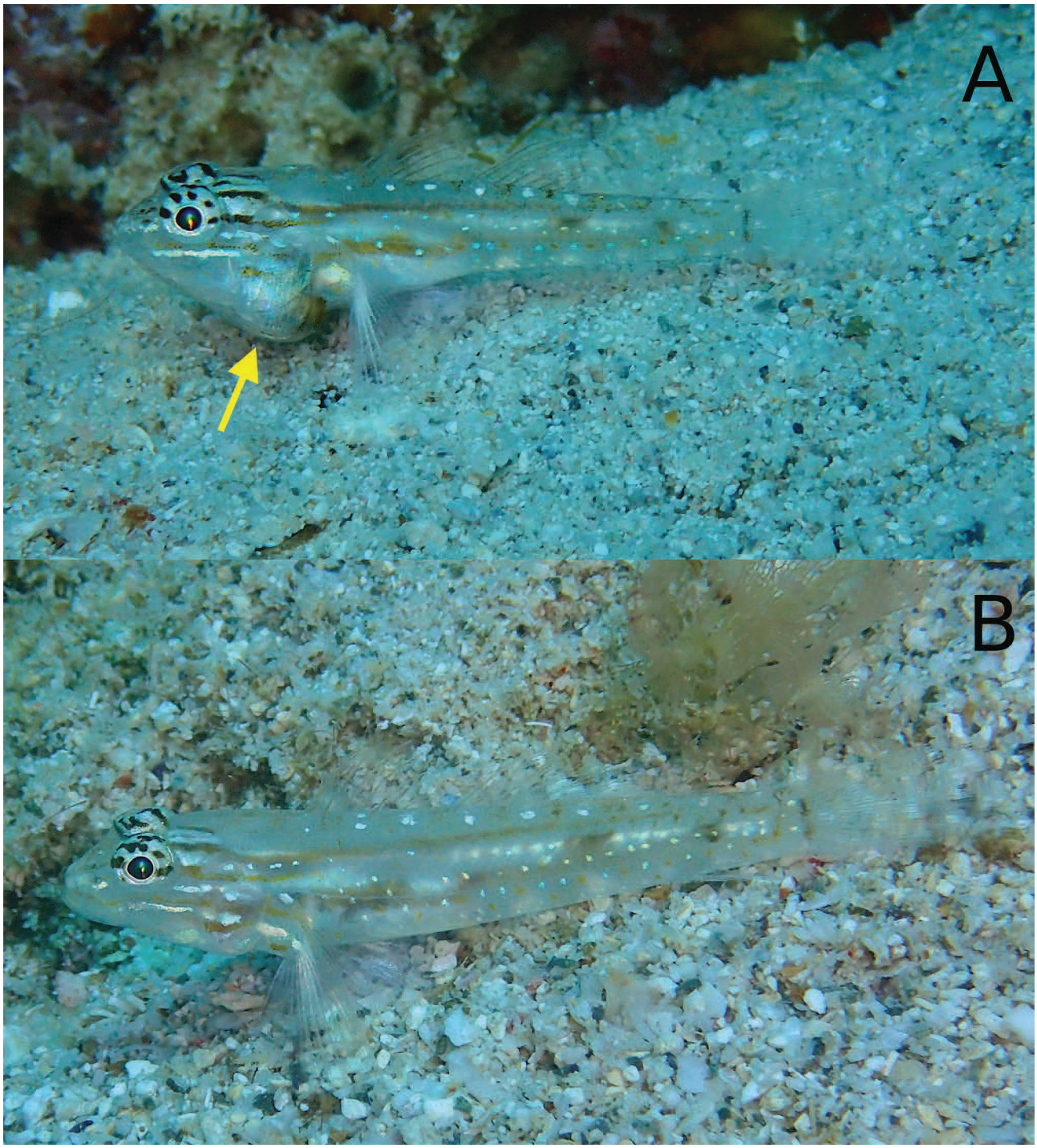

Fig. 1. Photographs of a goby infected with *P. tortugensis* (A) and an uninfected goby (B). The arrow in A indicates swelling of the opercular cavity due to infection.

Population-level effects of *P. tortugensis* on these goby hosts have been documented near Guana Island in the British Virgin Islands. In an observational field study, one of the common goby hosts (*C. venezuelae*) displayed slower growth, reduced fecundity, and suffered higher mortality when infected (Finley and Forrester 2003). The primary population-level influence of the parasite, however, is to compromise the gobies' ability to avoid the larger predatory fish that cause most goby deaths. Gobies flee to crevices in the reef when attacked and compete for refuges when they are short supply (Forrester and Steele 2004; Samhouri et al. 2009; Vance et al. 2010). Infection with

P. tortugensis diminished gobies' effectiveness as competitors for refuges and so strongly impacts their abundance (Forrester and Finley 2006; Forrester et al. 2019).

Objectives

This study had two sets of objectives. (1) We first tested for individual-level effects of *P. tortugensis* on *C. venezuelae* hypothesized to underly the population-level impacts previously reported. We documented the site(s) of attachment by *P. tortugensis*, patterns in the intensity of infection, and asked whether attachment damages host tissues at the infection site? We also asked whether infection alters the amount of energy allocated to reproduction (measured as gonad mass relative to body mass, Cole and Shapiro 1990) and to energy stored (measured as liver mass relative to body mass, Shul′man and Love 1999)? We also investigated whether infection influenced body condition (using body length- mass relationships and body mass-age relationships, Jakob et al. 1996)? (2) Second, we further describe host-parasite dynamics at the population and community level to assess the scope of their effects over time and over a broader geographic area. We documented the prevalence of *P. tortugensis* on its common *Coryphopterus* hosts*,* and also surveyed other potential hosts for infections, throughout the British Virgin Islands and US Virgin Islands. We also searched for *Coryphopterus* hosts with visual symptoms of infection throughout the Caribbean and documented changed over time in the prevalence of infections on these hosts near Guana Island.

Because we sampled naturally infected *Coryphopterus* hosts and correlated parasite presence with host responses, we cannot unambiguously isolate effects of parasitism per se. We acknowledge that other unmeasured factors, if correlated with parasite presence, might cause the responses we tested. Offsetting the limitations of this approach is the benefit of being able to observe and sample large numbers of infected hosts in a natural setting.

MATERIALS AND METHODS

Host collection and identification

This study includes data collected from 1993–2019 and revisions in classification of *Coryphopterus* hosts over this period affected the accuracy with which we could identify hosts. In the early years of our study, *Coryphopterus tortugae* (Jordan) and *C. venezuelae* were not considered separate from *C. glaucofraenum,* but DNA barcoding in the mid 2000s supported the validity of each as distinct species (Victor 2008; Baldwin et al. 2009). Although similar in

appearance, these three species can be distinguished morphologically (Baldwin and Robertson 2015; Victor 2015; Robertson and Van Tassell 2019), and we confirmed the identify of preserved specimens collected prior to 2008. Some host identifications made prior to 2008, such as those made visually by divers, could not be reevaluated, so in each component of the study described below we specify the level of specificity to which hosts are identified. Identification of the copepods as *P. tortugensis* based on morphology (Petrik-Finley 2005) was corroborated by the author of the species (Ju-shey Ho, University of California Long Beach, personal communication 2002) and also by DNA barcoding (Forrester et al., in review).

Attachment locations of *P. tortugensis* on *Coryphopterus* hosts

To determine if the parasitic copepod showed preference in attachment location on the host, a mix of parasitized *C. glaucofraenum* and *C. venezuelae* (n = 74) were collected from Muskmelon Bay and White Bay, near Guana Island in August 2001 and October 2002 (Fig. 2). Goby hosts for this and all other parts of the study were collected individually on SCUBA using hand nets and anaesthetic (Quinaldine). Captured gobies were placed directly into plastic bags and euthanized with an overdose of Quinaldine. No copepods were ever observed in the bags, suggesting that they remain attached to the hosts after collection. The external body surface, gill arches, branchial chamber and underside of the operculum were carefully searched for *P. tortugensis* and the attachment location of copepod recorded. To assess whether attachment location was differed between male and female copepods, or was affected by body size, copepods were sexed and visually assigned to size classes (Table 5).

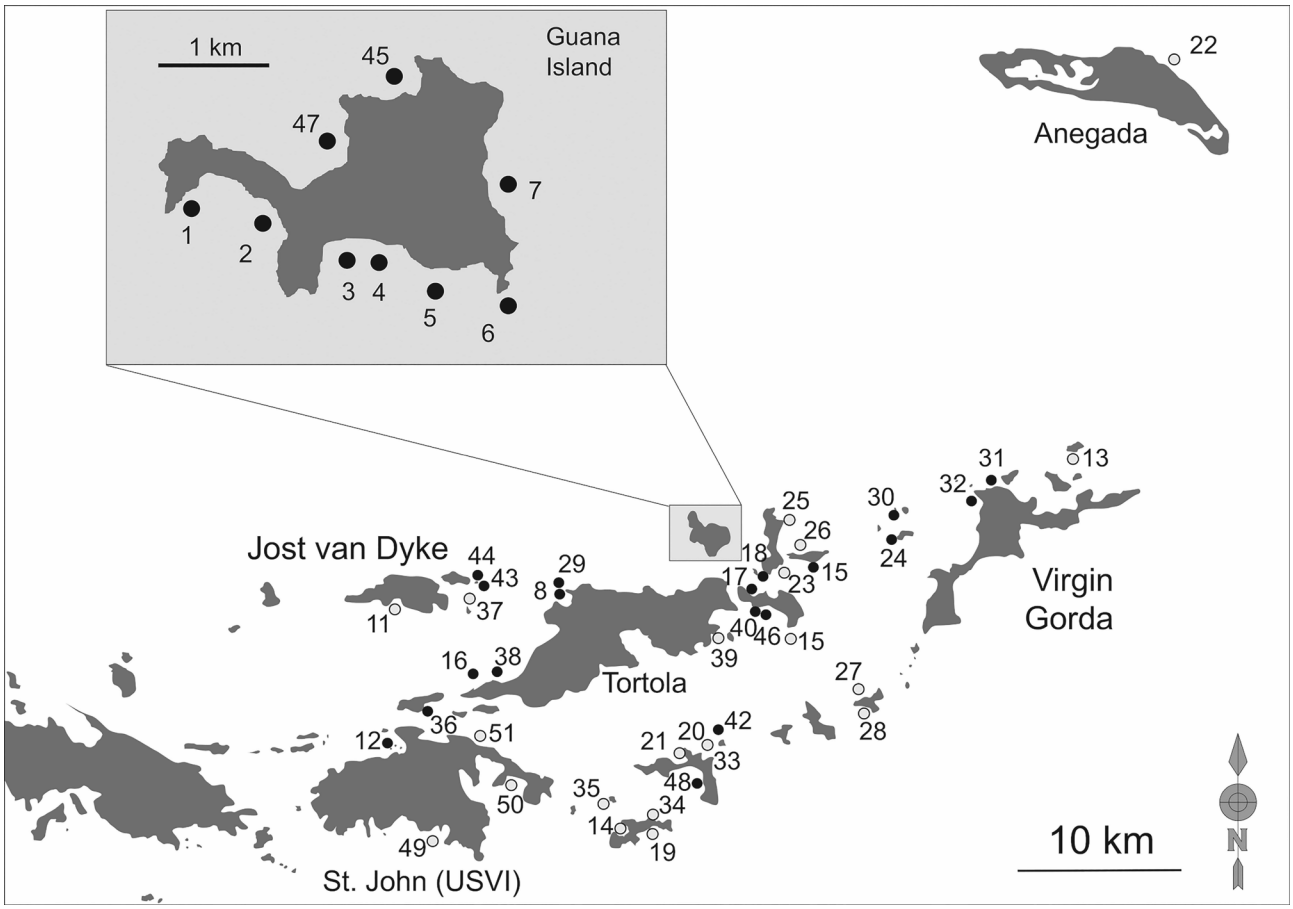

Fig. 2. Locations in the United States and British Virgin Islands where *Coryphopterus* were found infected with *Pharodes tortugensis*. Numbers correspond to sites listed in table 1.

Table 5. Body size and dimensions of *P. tortugensis*. Mean sizes (with 95% CI) are shown for female, male and juvenile of *P. tortugensis*. Females are grouped by reproductive status and males by size and p-values are reported for one-way ANOVAs comparing group means. See text for a detailed description of the morphological measurements

Females	Gravid (n = 52)	Not gravid (n = 53)	
	mean (95% CI)	mean (95% CI)	p-value
Body length	1.77 (1.70–1.85)	1.22 (1.15–1.29)	< 0.0001
Body width	2.35 (2.24–2.47)	1.34 (1.22–1.45)	< 0.0001
Head length	0.46 (0.45–0.47)	0.43 (0.42–0.45)	0.009
Lateral process length	1.33 (1.26–1.40)	0.80 (0.73–0.87)	< 0.0001
Egg sac length	1.68 (1.56–1.79)		
Egg sac width	1.03 (0.95–1.11)		
Males	Small (n = 103)	Large (n = 203)	
	mean (95% CI)	mean (95% CI)	p-value
Body length	0.33 (0.31–0.35)	0.70 (0.68–0.71)	< 0.0001
Body width	0.24 (0.22–0.26)	0.54 (0.53–0.55)	< 0.0001
Juveniles (n = 34)	mean (95% CI)		
Body length	0.18 (0.15–0.22)		
Body width	0.11 (0.09–0.14)		

We divided female copepods into three classes based on their size and morphology: immature transforming, mature non-gravid, and mature gravid. Transforming females are those that are metamorphosing from the typical copepod morphology to the modified fleshy adult female.

During transformation, the lateral processes extend from the trunk, the caudal process elongate, and the head becomes more distinct. Mature females were distinguished from transforming females by their possession of distinct and well-developed heads, and lateral and cephalic processes (Fig. 2). Gravid females were distinguished by their possession of two large egg sacs, which extended from the caudal process (Fig. 3B). Transforming females were assumed to be immature because they never possessed egg sacs. Male copepods were found in several locations, so a Chi^2 test was used to test whether the frequency of attachment differed among these locations.

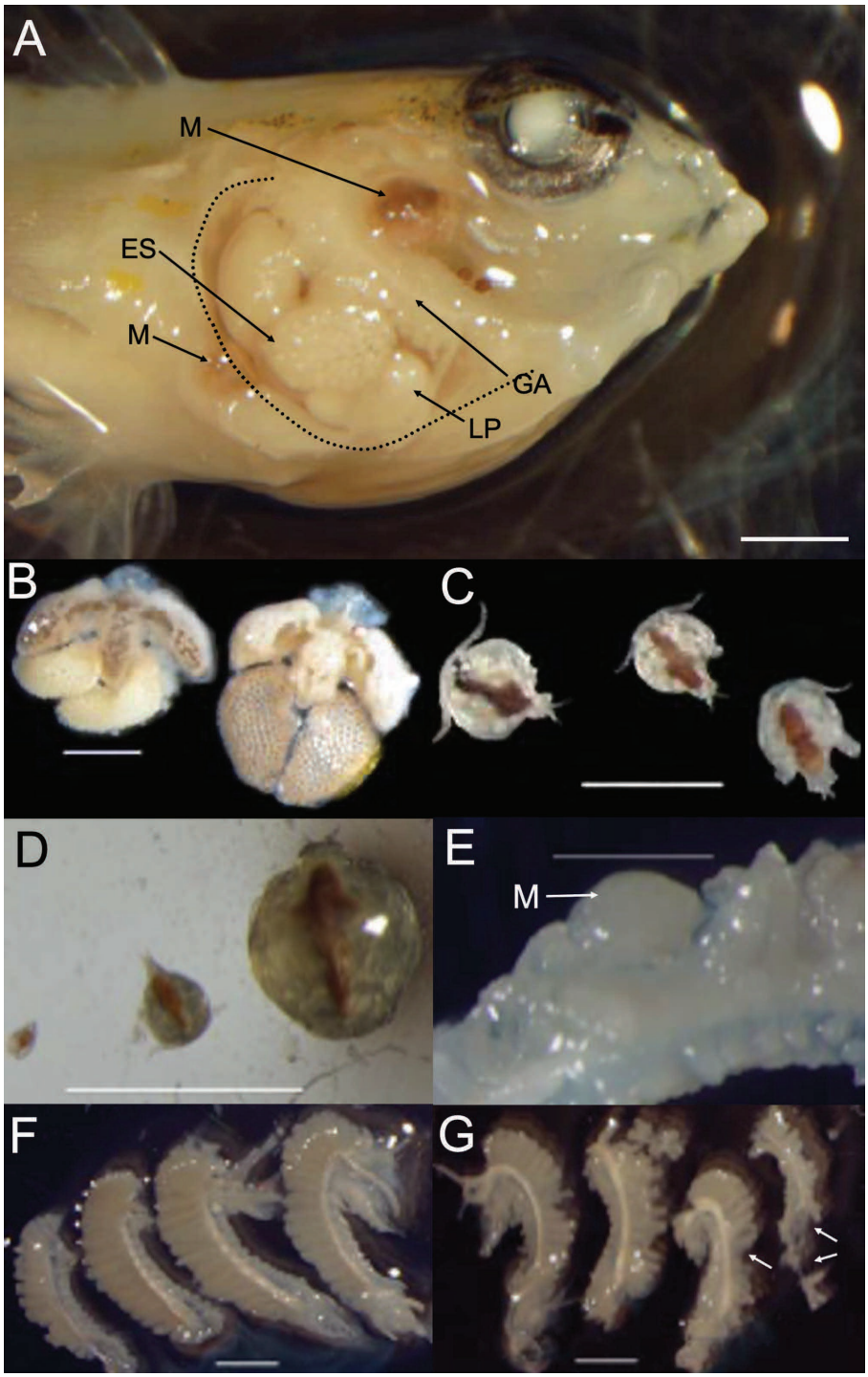

Fig. 3. (A–G). Photographs detailing infection by *P. tortugensis* in gobies: the size and morphology of male and female copepods, and gill damage in parasitized fish. Scale bars are = 1 mm in all photos. (A) Profile of an infected *C. glaucofraenum* with operculum removed. Dotted line indicates perimeter measurement. Abbreviations are: GA: 1st gill arch, M: placement of males on the gill arch and on perimeter of gill cavity wall, LP: lateral process of gravid female copepod attached to host gill cavity wall, ES: egg sac of gravid female copepod. (B) Two gravid female copepods. (C) Three large male copepods. (D) Size comparison of juvenile (left), small male (center), and large male (right). (E) Gill arch with a male copepod (M) attached. (F) Display of gill arches 4 – 1 (L to R) from an unparasitized goby. (G) Gill arches 1 – 4 (L to R) from a parasitized goby. Damaged and missing filaments are indicated with arrows on the 3rd and 4th arches. Some filaments are also missing, and less severe damage overall is seen in the 1st and 2nd arches.

Infection intensity of *P. tortugensis* on *C. venezuelae*

To describe patterns in the intensity of infections (sensu Bush et al. 1997), a collection of 331 *C. venezuelae* was made in 2004 (n = 284) and 2018 (n = 47). These gobies were collected at random, without regard for size or infection symptoms, from the White Bay site (Fig. 2). The gobies were measured in standard length (SL, the distance from the tip of the nose to the end of the caudal peduncle) and grouped into four size categories: < 15, 15–20, 20–25, and 25–30 mm SL, to examine whether the intensity of infection differed among size categories. Each goby was then searched for copepods. To test for over-dispersion, we examined both the variance to mean ratio of the number of copepods per fish and tested if the frequency of infection intensity fit a negative binomial distribution (following the methods described by Krebs 1999).

Size-distributions of male and female *P. tortugensis*

To describe the size distribution of *P. tortugensis*, we took digital photographs of male and female copepods taken from infected *C. venezuelae* collected in 2004 and measured them using imaging software (ImageJ version 2.10; Schindelin et al. 2012). On male copepods, body length was defined as the combined length of the cephalothorax and genital segment, and body width was the greatest distance across the cephalothorax. For female copepods, body length was the distance from the tip of the head between the antennae to the end of the caudal process, and body width was a linear distance between the tips of the lateral processes (anatomical terminology follows Ho 1971a). We also measured the maximum length and width of egg sacs from gravid female copepods.

Gill pathology of parasitized *C. venezuelae*

To describe damage to the gill arches of *C. venezuelae* by *P. tortugensis*, we dissected parasitized (n = 94) and unparasitized (n = 190) gobies collected in 2004 and inspected their gill arches. Damage to the gill arches was defined as: compression of the gill filaments, mucus completely covering filaments, and filaments missing from the gill arches and the percent of the arch damaged was quantified visually. Our preliminary impression was that damage was intensified when infections included female copepods, rather than just males and/or juveniles. We therefore divided the gobies into these two groupings and used analysis of variance (ANOVA) to test if they differed in percent of branchial damage (unparasitized gobies were included as a third control group). Prior to this and other analyses using linear models, we used Q-Q plots and plots of the residuals versus predicted values to confirm that the data met the assumptions of the model. For this

analysis, data were square root arcsine transformed prior to analysis to meet the assumptions (following Zar 1996).

To determine if female copepods enlarge the branchial chamber of parasitized gobies, digital photographs were taken of the right and/or left branchial cavities of some parasitized and unparasitized bridled gobies (n = 8) after the operculum was removed (Fig. 3). Imaging software (ImageJ version 2.10; Schindelin et al. 2012) was used to measure the perimeter of the branchial cavity in gobies parasitized with female copepods and compared to the perimeter of unparasitized gobies (see Fig. 3 for a diagram illustrating the perimeter measured). To adjust for the fact that the branchial chamber size should be a function of the fish size; differences in branchial chamber perimeter were tested using analysis of covariance (ANCOVA) with fish length as a covariate and infection (yes or no) as a categorical variable.

Effects of *P. tortugensis* on host body condition

To assess the effect of *P. tortugensis* on host body condition and energy allocation, a mix of *C. glaucofraenum* and *C. venezuelae* were collected from Muskmelon Bay and White Bay near Guana Island in from 2001–2003 (Fig. 2). The gobies were fixed in 70% alcohol, measured (SL) and sexed by examining the genital papilla (Cole and Shapiro 1990, 1992). Individuals spanning the size range at which most infections occurred were used in this analysis (n = 163, 12.2 – 33.7 mm SL). These gobies change sex from female to male (protogynous hermaphroditism), and individuals of this size range comprise mainly juveniles and females (Cole and Shapiro 1990, 1992). Excluding larger individuals because they were rarely infected by *P. tortugensis* thus also excluded most males from the sample.

The otoliths (lapilli) were removed from each goby and, after clearing in immersion oil, the post-settlement age (days) was determined by counting the daily growth rings formed after the mark on the otolith that indicates settlement to the reef (Steele and Forrester 2002). The copepods were removed from parasitized gobies, dried at 60°C until a constant mass was achieved (at least 1 h) then weighed in mg to 0.001 mg. The liver and gonads were removed from each goby, and these organs plus the body tissue (minus the alimentary tract) were each dried and weighed. Total goby body mass was calculated as the sum of liver, gonad, and body tissue mass.

To assess the effect of *P. tortugensis* on body condition, we measured total body mass as a function of body length (*SL*) (Ogle 2016). To assess the effect of *P. tortugensis* on investment in energy reserves and reproduction allocation, we measured liver (*LM*) and gonad (*GM*) mass respectively as a function of total body mass (*BM*). To explore whether changes in condition might affect growth we examined body mass as a function of post-settlement age (*A*).

Fish mass-length (*BM* vs. *SL*) relationships are non-linear and are typically modeled as a power function of the form

$$BM_i = aSL_i^b \, e^{c_i}$$

where a and b are constants and c_i is the multiplicative error term for the ith fish (Ogle 2016). This relationship was linearized as follows

$$\log(BM_i) = \log(a) + b\log(SL_i) + c_i$$

and analysis of covariance (ANCOVA) was used to test the effect of parasitic infection (*IN*), a categorical variable (infected or not), on the transformed relationship as follows

$$\log(BM_i) = \log(a) + b\log(SL_i) + d(IN) + eIN \times \log(SL_i) + c_i$$

Inspection of the data suggested that the other relationships of interest (*LM* vs. *BM*, *GM* vs. *BM*, and *BM* vs. *A*), also resembled power functions rather than linear functions. ANCOVA models of the same form as the *BM* vs. *SL* model were thus used to test for effects of parasitism on these other relationships.

Because the effects of some macroparasites are related to the number of parasites per host, rather than just parasite presence, we tested whether infection abundance (sensu Bush et al. 1997) was a better predictor of host impact than parasite presence. Copepods varied greatly in size, so we used the combined mass of copepods on a host, rather than the number of copepods, as our index of infection abundance. For each of the relationships just described (*BM* vs. *SL, LM* vs. *BM*, *GM* vs. *BM*, and *BM* vs. *A*), we substituted infection intensity (*II*) for parasite presence (*IN*) in the above linear models. Because infection abundance (*II*) is a continuous variable and infection presence (*IN*) is categorical, the model becomes a multiple regression rather than ANCOVA. For each pair of models (*II* vs. *IN*), we used Akaike's Information Criterion (AICc) as a measure of relative model fit, and models differing in AIC by < 6 were judged to have similar support in the data (Richards 2005).

Spatial and temporal patterns in the distribution of hosts infected with *P. tortugensis*

To document the spatial distribution of *P. tortugensis* infections on common *Coryphopterus* hosts in the British and US Virgin Islands, we performed visual surveys on SCUBA at 52 sites in the area (Table 1; Fig. 2). Individuals were classified as parasitized or not based on visual symptoms of infection. These counts represent the combined abundance of *C. glaucofraenum*, *C. venezuelae* and *C. tortugae*. We made quantitative surveys at 35 sites, in which all gobies within transe cts (0.5 × 4 m) were inspected and counted. At 17 other sites, we made less rigorous qualitative surveys in which gobies encountered were visually inspected and *P. tortugensis* was noted the either present or absent at the site.

Table 1. Geographic pattern of infections of *P. tortugensis* on *Coryphopterus* in the British Virgin Islands (BVI) and U. S. Virgin Islands (USVI). Data are prevalence from transect counts (mean % with number of transects in brackets) or presence/absence from visual searches. Dashes (-) indicate no data for a given site and year. Map numbers correspond to sites in figure 2

Island Group	Island	Site name	Latitude (N)	Longitude (W)	Map #	2001	2002	2003	2004	2008
BVI	Guana Island	Muskmelon Bay	18° 28.9	64° 34.78	1	9% (7)	17% (8)	Yes	Yes	-
BVI	Guana Island	Crab Cove	18° 28.79	64° 34.70	2	13% (10)	13% (10)	Yes	Yes	-
BVI	Guana Island	White Bay Dock	18° 28.54	64° 34.65	3	19% (19)	Yes	Yes	Yes	-
BVI	Guana Island	Harris Gut			4	21% (9)	Yes	Yes	Yes	-
BVI	Guana Island	White Bay	18° 28.13	64° 34.41	5	21% (19)	12% (15)	Yes	16% (5)	-
BVI	Guana Island	Monkey Point	18° 27.98	64° 34.30	6	25% (10)	7% (11)	Yes	Yes	-
BVI	Guana Island	Bigelow Beach			7	1% (20)	5% (8)	Yes	Yes	-
BVI	Tortola	Brewer's Bay Inside			8	14% (18)	-	Yes	Yes	-
BVI	Little Camanoe	East Bay	18° 27.42	64° 32.15	10	-	4% (10)	-	-	-
BVI	Jost van Dyke	Great Bay			11	-	No	-	-	-
USVI	St John	Cinnamon Bay			12	-	Yes	-	-	-
BVI	Necker Island	Necker Island	18° 31.45	64° 21.61	13	-	0% (10)	-	-	-
BVI	Norman Island	The Bight			14	-	0% (10)	-	-	-
BVI	Scrub Island	South	18° 27.92	64° 31.18	15	-	6% (10)	-	No	-
BVI	Tortola	Smuggler's Cove			16	-	-	Yes	-	-
BVI	Beef Island	Long Bay			17	-	-	Yes	Yes	-
BVI	Beef Island	Airport Runway Dock			18	-	-	Yes	Yes	-
BVI	Norman Island	Money Bay			19	-	-	No	-	-
BVI	Peter Island	Deadman Bay			20	-	-	No	-	-
BVI	Peter Island	Little Harbour			21	-	-	No	-	-
BVI	Anegada	Loblolly Bay			22	-	-	No	-	-
BVI	Great Camanoe	Diamond Reef			23	-	-	No	-	-
BVI	Great Dog	Coral Gardens	18° 28.93	64° 27.70	24	-	-	-	2% (8)	-
BVI	Great Camanoe	Northeast Point			25	-	-	-	No	-
BVI	Scrub Island	North	18° 28.25	64° 31.02	26	-	-	-	0% (5)	-
BVI	Ginger Island	North	18° 23.32	64° 29.14	27	-	-	-	0% (10)	-
BVI	Ginger Island	South	18° 23.19	64° 28.90	28	-	-	-	0% (9)	-
BVI	Tortola	Brewer's Bay Outside	18° 26.90	64° 39.21	29	-	-	-	17% (7)	-
BVI	George Dog	Bronco Billy	18° 29.49	64° 27.52	30	-	-	-	2% (4)	-
BVI	Mosquito Island	South	18° 30.57	64° 24.05	31	-	-	-	1% (3)	-
BVI	Virgin Gorda	Mountain Point	18° 30.06	64° 24.94	32	-	-	-	2% (5)	-
BVI	Dead Chest	West	18° 22.12	64° 33.83	33	-	-	-	4% (5)	-
BVI	Norman Island	North	18° 19.42	64° 36.65	34	-	-	-	0% (5)	-
BVI	Pelican Island	Reef Check Site			35	-	-	-	0% (5)	-
BVI	Great Thatch	South	18° 22.923	64° 44.37	36	-	-	-	2% (9)	-

BVI	Sandy Cay	North	18° 26.245	64° 42.79	37	-	-	-	0% (5)	-
BVI	Tortola	Beaumont Point	18° 23.99	64° 41.80	38	-	-	-	10% (10)	-
BVI	Buck Island	West Bay			39	-	-	-	No	-
BVI	Beef Island	Hans Creek 1			40	-	-	-	No	-
BVI	Beef Island	Airport	18° 26.22	64° 32.76	41	-	-	-	-	8% (5)
BVI	Dead chest	East	18° 22.07	64 33.75	42	-	-	-	-	6% (5)
BVI	Green Cay	Green Cay 1	18° 27.32	64° 42.49	43	-	-	-	-	3% (6)
BVI	Green Cay	Green cay 2	18° 45.39	64° 70.99	44	-	-	-	-	1% (6)
BVI	Guana Island	Grand Ghut	18° 28.79	64° 33.70	45	-	-	-	-	6% (5)
BVI	Beef Island	Han's Creek 2	18° 26.21	64° 31.90	46	-	-	-	-	2% (5)
BVI	Guana Island	North Bay	18° 28.71	64° 34.64	47	-	-	-	-	5% (6)
BVI	Peter Island	White Bay	18° 21.43	64° 35.42	48	-	-	-	-	0% (5)
USVI	St John	Lameshur Bay			49	-	Yes	-	Yes	-
USVI	St John	Round Bay			50	-	-	-	Yes	-
USVI	St John	Brown Bay			51	-	-	-	Yes	-
USVI	St Croix	East End			52	-	No	-	No	-

To document changes over time in the prevalence of infection, repeated estimates were made from 1993–2019 at a BVI site near Guana Island (Harris Ghut, Table 1, and Fig. 2). *C. venezuelae* from this site were classified as parasitized or not based on visual symptoms of infection (Table 2). Some estimates were based on inspection of individuals during underwater surveys, whereas other individuals were captured using hand nets for other experiments and inspected underwater while in the net prior to release back into the field.

Table 2. Prevalence of *P. tortugensis* on *C. venezuelae* at Harris Ghut, near Guana Island based on visual inspections of hosts for signs of infection

Year	# hosts inspected	# infected	Prevalence
1993	99	3	3%
1994	121	3	2%
1995	114	4	4%
1996	95	12	13%
1997	71	18	25%
1998	0	-	-
1999	0	-	-
2000	138	19	14%
2001	126	27	21%
2002	0	-	-
2003	0	-	-
2004	237	56	24%
2005	97	12	12%
2006	63	12	19%
2007	88	12	14%
2008	0	-	-
2009	19	2	11%
2010	34	5	15%
2011	74	23	31%
2012	0	-	-
2013	0	-	-
2014	45	5	11%
2015	101	12	12%
2016	154	21	14%
2017	0	-	-
2018	162	29	18%
2019	192	29	15%

To document the broader distribution of *P. tortugensis* infections on three common *Coryphopterus* hosts (*C. glaucofraenum*, *C. venezuelae* and *C. tortugae*), visual surveys were performed at 16 other sites throughout the Caribbean (Table 3). Hosts were screened visually for symptoms on SCUBA as encountered. Parasitized hosts were counted individually, and the total number of hosts screened per location was recorded to the nearest 10 ($n \approx 4900$; Table 3).

Table 3. Hosts of *P. tortugensis* screened for infections at other Caribbean locations. Three hosts (*C. glaucofraenum, C. tortugae* and *C. venezuelae*) were inspected visually underwater for symptoms of infection. Hosts were not identified to species and data are pooled; the number of hosts screened is given to the nearest 10

Location	Years	# hosts inspected	Prevalence
Barbados	2007, 2014	110	0
Bahamas	1995–2006	2200	0.005
Belize	2006, 2016	340	0
Bonaire	2006, 2008, 2014–2019	480	0
Curacao	2008	120	0
Dominica	2008–2009	160	0
Grenada	2010	120	0
Honduras	2019	40	0
Jamaica	1994–1999, 2002	330	0.006
Mexico	2002	140	0
Puerto Rico	2012	70	0
Saba	2008, 2010	160	0
St. Eustatius	2008	150	0
St. Lucia	2007–2008	200	0
St. Vincent	2007	140	0
Tobago	2008, 2010	110	0

In addition to the four species of *Coryphopterus* we studied, *P. tortugensis* has been reported from 11 species of fish host at other locations in the western Atlantic (NMNH 2020, WORMS 2020). Most of these other hosts are gobies (Gobiidae Cuvier) or blennies (Blenniidae Rafinesque). We therefore sought to identify potential additional hosts of *P. tortugensis* from these families in the British Virgin Islands (Table 4). Some potential hosts were collected and their external body surface, gill arches, branchial chamber and underside of the operculum were carefully searched for *P. tortugensis*. Other hosts were inspected visually on SCUBA for the distended operculum symptomatic of infection.

Table 4. Possible alternative hosts of *P. tortugensis* screened for infections in the BVI. No signs of infection by *P. tortugensis* were observed on any of these hosts. Host fishes previously reported to host *P. tortugensis* (known) or related ecologically and taxonomically to known hosts (suspected), were either dissected or inspected visually underwater for symptoms of infection. Size ranges for hosts inspected visually are estimates

Host status	Host Family	Host species	Host common name	Dissected		Visual inspection	
				n	Size range (mm SL)	*n*	Size range (mm SL)
Suspected	Gobiidae	*Coryphopterus personatus*	masked goby	9	7–19	34	8–20
Suspected	Gobiidae	*Coryphopterus hyalinus*	glass goby	11	7–19	43	8–20
Suspected	Gobiidae	*Coryphopterus lipernes*	peppermint goby	-	-	22	10–20
Suspected	Gobiidae	*Coryphopterus alloides*	barfin goby	-	-	13	10–30
Suspected	Gobiidae	*Gnatholepis thompsoni*	goldspot goby	14	13–38	121	10–40
Suspected	Gobiidae	*Tigrigobius multifasciatus*	greenbanded goby	-	-	16	10–25
Known	Gobiidae	*Bathygobius soporator*	frillfin goby	-	-	7	20–50
Known	Gobiidae	*Tigrigobius saucrus*	leopard goby	-	-	12	8–15
Known	Gobiidae	*Elacatinus chancei*	shortstripe goby	-	-	17	10–30
Known	Gobiidae	*Elacatinus evelynae*	sharknose goby	-	-	22	10–30
Known	Blenniidae	*Scartella cristata*	molly miller	-		5	15–30
Suspected	Blenniidae	*Malacoctenus boehlkei*	diamond blenny	9	26–43	10	15–30
Suspected	Blenniidae	*Malacoctenus macropus*	rosy blenny	18	16–31	12	15–25
Suspected	Blenniidae	*Parablennius marmoreus*	seaweed blenny	49	16–43	-	-

RESULTS

Attachment locations of *P. tortugensis*

P. tortugensis was found only in or around the branchial chamber of *C. venezuelae* and *C. glaucofraenum*, even though other possible sites of infection were searched thoroughly. Attachment locations differed according to copepod sex and body size. *P. tortugensis* can be quite large (Table 5), especially considering that most *Coryphopterus* hosts are < 30 mm in length. Male copepods were generally smaller than females and their size distribution was clearly bimodal (Fig. 4). For that reason, males were divided into two size categories, large and small (Table 5, Fig. 3D), using the break point between the two modes (0.45 mm).

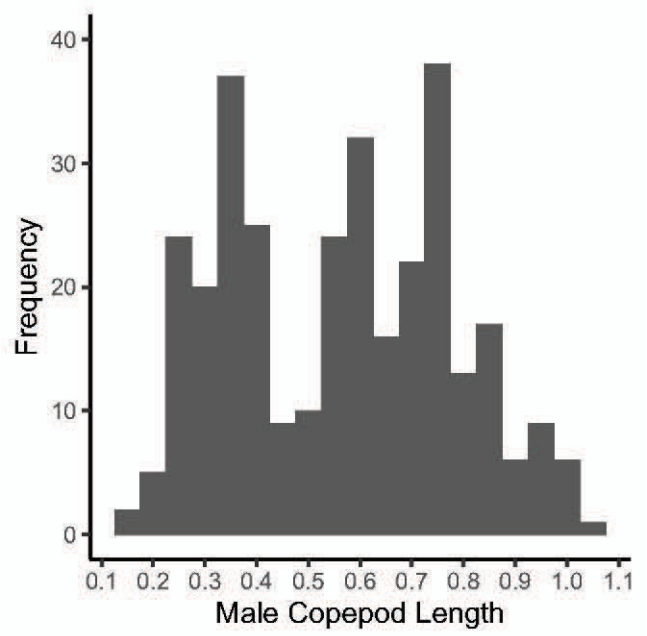

Fig. 4. Size-frequency distribution of male *P. tortugensis* body length (measured in mm).

Virtually all female copepods inspected (116 out of 119) were attached to the ventral surface of the branchial chamber, below the gill arch, with their heads burrowed into the body wall. One of the 3 remaining females was attached to the underside of the operculum, one was just outside of the branchial chamber on the body of the goby, and the last was attached to a gill arch. All three of these females were small and still in the transforming phase. Most of the gobies collected in 2004 were infected by two female copepods, one in each gill chamber, and very few infections consisted of three female copepods (Fig. 5A).

Male copepods were found attached at six locations: on the ventral surface of the branchial chamber, on the underside of the operculum, and on each of the four gill arches (Fig. 5B). A Pearson's χ^2 goodness of fit test showed that the males were not evenly distributed among these locations ($\chi^2 = 114.28$, *d.f.* = 5, $p < 0.001$). Large males were attached to the ventral surface of the branchial chamber, or gill arches 1 and 2, and so were in closer proximity to female copepods than were small males. The frequency of attachment of large males did not differ among these three locations ($\chi^2 = 2.881$, *d.f.* = 2, $p = 0.263$). In addition to these three locations, small males also attached to gill arch 3, the underside of the operculum, and to gill arch four. Sequential removal of these three sites from the original goodness of fit analysis confirmed that these peripheral locations were less frequently occupied than the locations closer to the females ($p < 0.032$).

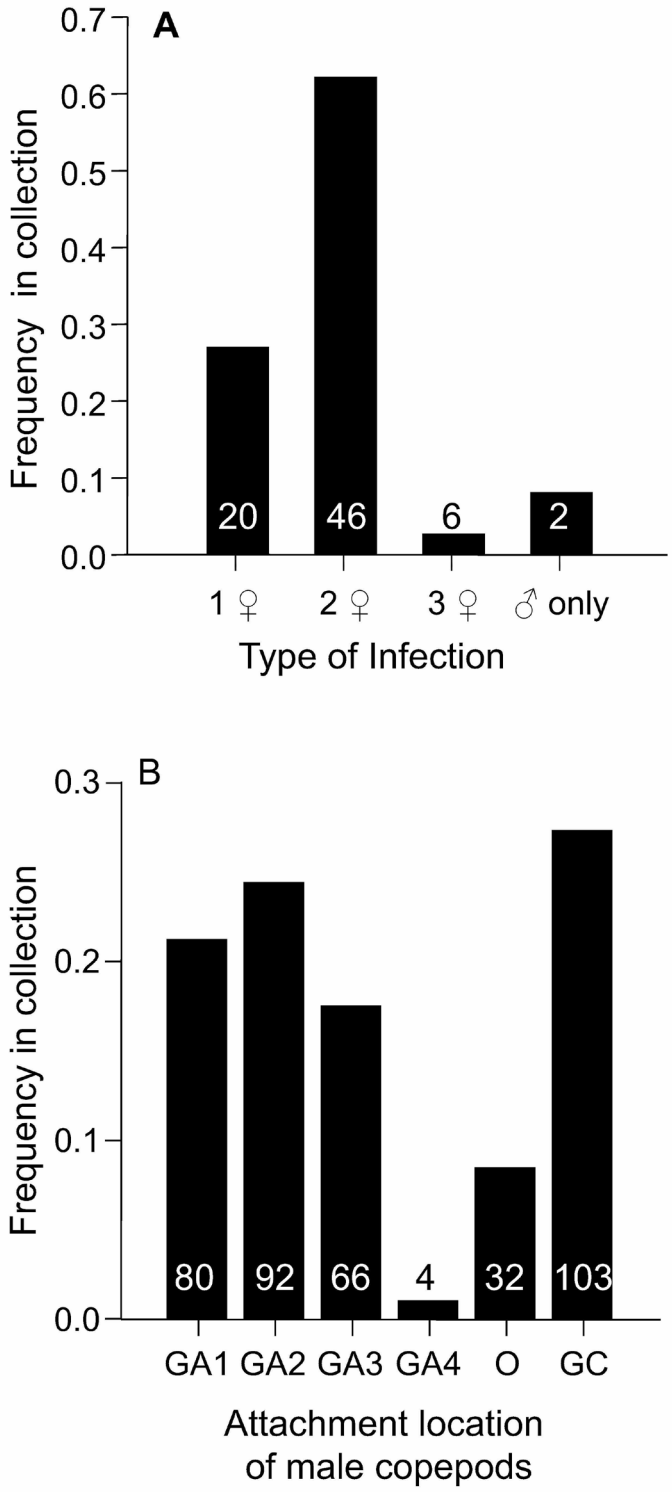

Fig. 5. The frequency of infections with different numbers of female copepods (1, 2 or 3) and infections with only male copepods (A), and attachment locations of male copepods (B) in the branchial chambers of *C. Venezuelae*. Attachment locations are: GA1 = gill arch 1, GA2 = gill arch 2, GA3 = gill arch 3, GA4 = gill arch 4, O = underside of the operculum, and on each of the four gill arches, GC = ventral surface of the branchial chamber. Sample size is indicated with each bar.

Prevalence and intensity of infections

Thirty-two percent of *C. venezuelae* sampled at random in 2004 were infected with *P. tortugensis.* The number of copepods per host ranged from 1–17 (mean = 4.7). Roughly a quarter of the copepods (25.4%) were female, and the number of females per host was much lower on average (mean = 0.39 per host) and less variable (range = 1–3) than the number of males and juveniles (mean = 3.7, range = 1–15).

As is typical of macroparasite infections, *P. tortugensis* was over dispersed within the host population (Fig. 6). Overdispersion was indicated by a high variance to mean ratio of 5.4 and the fact that distribution of the copepods among gobies was well described by the negative binomial distribution ($k = 0.188$; χ^2 goodness of fit = 0.13, $d.f. = 15$, $p > 0.9$, Fit) and did not conform to the Poisson distribution expected if infections were distributed at random (χ^2 goodness of fit = 1733, $d.f. = 15$, $p < 0.001$). The distribution of infection intensity was examined in four size classes of parasitized gobies to determine if infection intensity changed with fish size (Fig. 7). No infections were observed in bridled gobies < 10.0 mm SL and > 29.4 mm SL, which is consistent with previous collections ($n = 1213$, Petrik-Finley 2005). Within the size-classes that were infected, infection intensity appeared to increase with host body size (Fig. 6).

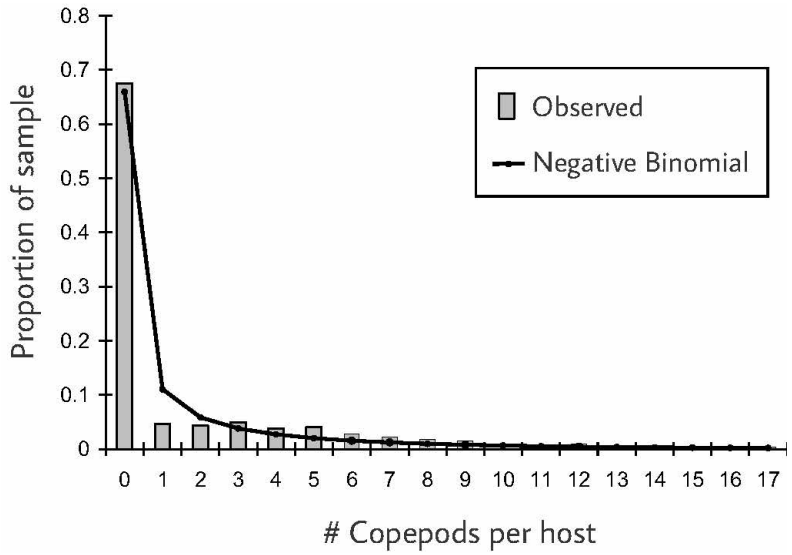

Fig. 6. The frequency distribution of *P. tortugensis* among goby hosts. The observed distribution is shown alongside the negative binomial distribution.

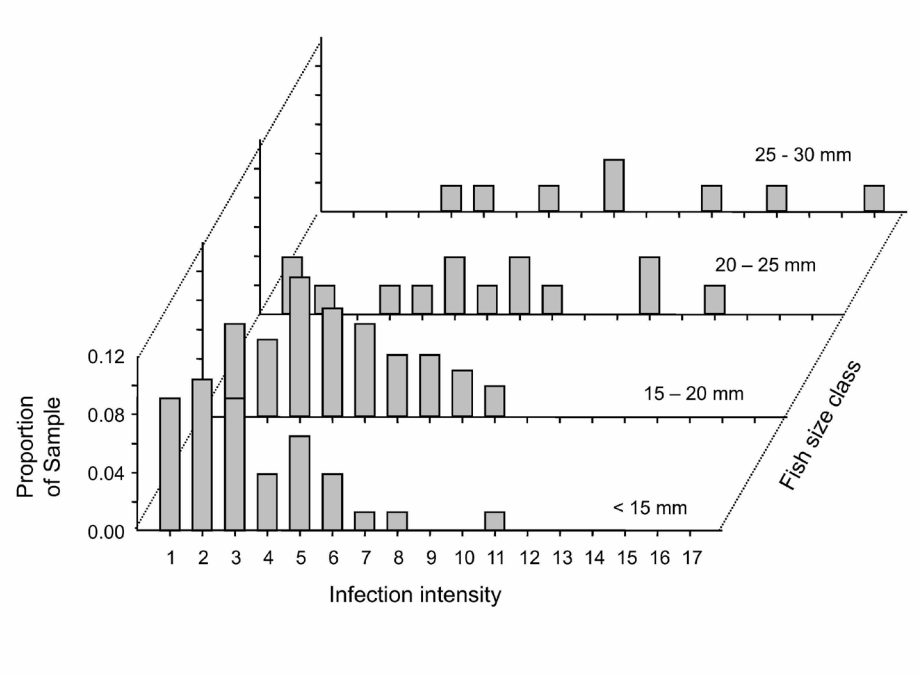

Fig. 7. Infection intensity of *P. tortugensis* in four size classes of goby (*C. glaucofraenum* and *C. venezuelae* pooled).

Gill pathology in parasitized bridled gobies

Infection by *P. tortugensis* caused various symptoms of damage to the gill cavity and respiratory surface (Fig. 3). Consistent with our ability to visually diagnose infections based on swelling of the operculum, infected individuals had measurably enlarged gill cavities. The slope of the relationship between the length of the gill cavity perimeter and body size was similar for infected and uninfected fish (ANCOVA: $F_{1,7} = 0.90$, $p = 0.37$), but at a given body size, parasitized gobies had enlarged gill chambers relative to uninfected ones (ANCOVA: $F_{1,8} = 23.74$, $p = 0.001$; Fig. 8).

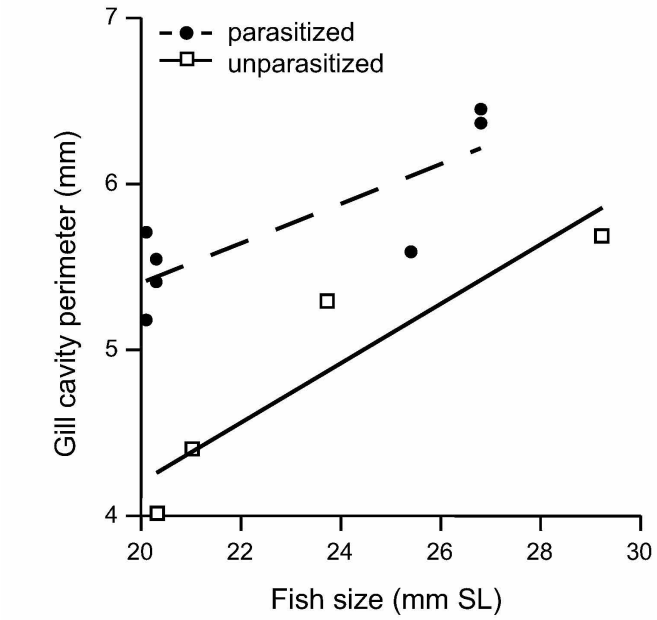

Fig. 8. Gill cavity perimeter as a function of body size in parasitized and unparasitized *C. venezuelae*.

Inside the gill cavity, all infected fish had gill filaments that were either visibly damaged, atrophied or missing and large portions of the gill arches and filaments were covered in mucus. The extent of damage depended on the intensity of infection and presence of female copepods. Males and juveniles are mobile, but cause damage at the locations on gills there they attach with their hooked antennae (Fig. 3E). Their presence results in damaged or atrophied filaments (Fig. 3E–G). Infections with only males/juveniles resulted in damage to 7% (± 0.6% SE) of the gill arches on average (for comparison uninfected gobies averaged 0.2 % damage). Mature female copepods, however, caused far more severe damage. When a mature female occupied the branchial chamber, gill filaments were compressed where its body pressed against the filaments and arches (Fig. 2A) and, on average, 21% of the gill arch was damaged (± 0.5% SE). These differences in percent damage between males, females, and uninfected fish were significant (ANOVA $F_{2,281} = 165.623$, $p < 0.001$; Tukey's pairwise posthoc tests, MSE = 72.052, *d.f.* = 281, p always < 0.001). In addition, the head of each female copepod created a cavernous wound in the ventral surface of the gill cavity wall at the site of attachment at least as large and deep as the size of the female head (Table 5). Some larger uninfected gobies possessed a crater in the ventral surface of the branchial chamber covered in mucus and damage to the gills similar that observed in infected individuals, implying that they had recently shed an infection.

Body condition and investment in energy stores and reproduction

Body condition, measured as the relationship between body mass and length, was virtually identical for infected and uninfected *C. venezuelae* (Fig. 9). ANCOVA indicated that neither the constant ($F_{1,161} = 1.52$, $p = 0.22$) nor the exponent ($F_{1,161} = 0.99$, $p = 0.32$) of the mass-length relationship was significantly affected by parasitic infection. The combined mass of copepods on a host was a non-trivial percentage of overall host mass (mean = 1.64%, SD = 1.51%). Using this index of infection intensity as the measure of parasite impact improved model fit (Table 6), but the impact of parasitism remaining non-significant.

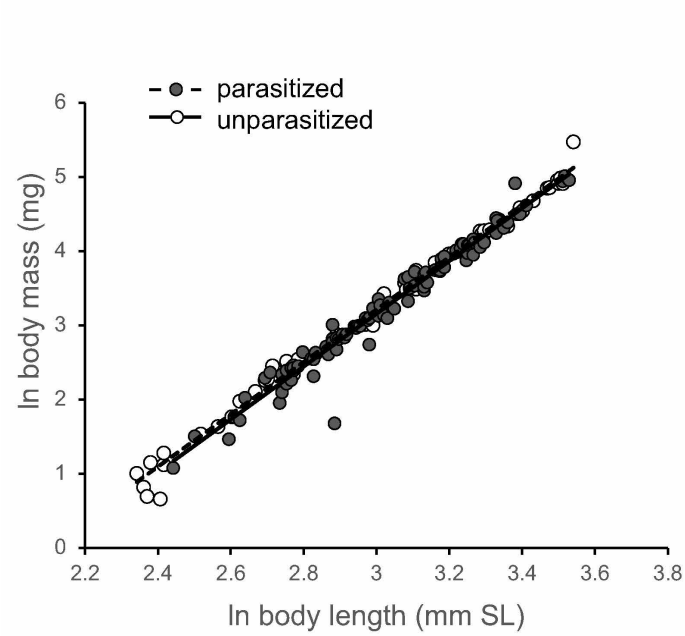

Fig. 9. Weight-length relationship in parasitized and unparasitized gobies (*C. glaucofraenum* and *C. venezuelae* pooled).

Table 6. Relative fit (measured using AIC) of models using parasite presence (*IN*) and the intensity of infection (*II*) as alternate measures of parasite impact. Models predict the impact of parasitism on a) host condition, b) investment in energy stores, c) investment in reproduction, and d) growth respectively. * Indicates better fitting model

Relationship	Index of parasite impact	
	Presence (*IN*)	Intensity (*II*)
a) Body mass (*BM*) vs. body length (*SL*)	-149.9*	-121.0
b) Liver mass (*LM*) vs. body mass (*BM*)	132.6	126.9
c) Gonad mass (*GM*) vs. body mass (*BM*)	152.4	142.9*
d) Body mass (*BM*) vs. age (*A*)	91.1	88.3

The mass of liver and gonads were a small fraction of overall body mass (< 5%) and, despite not influencing overall condition, parasitism affected both organs. Liver mass increased with body mass at a similar rate in infected and uninfected *C. venezuelae* (ANCOVA: $F_{1,147} = 0.13$, $p = 0.72$), but at any given size, infected individuals had larger livers (ANCOVA: $F_{1,147} = 3.94$, $p = 0.049$; Fig.

10). There was no improvement in model fit from using infection intensity (copepod mass), rather than parasite presence, as the measure of parasite impact (Table 6)

Gonad mass displayed a different overall pattern; the gonads of uninfected gobies progressively increased in mass as they increased in body mass, whereas gonads of infected gobies showed no tendency to increase in mass as they grew larger (ANCOVA interaction term: $F_{1,43}$ = 10.55, p = 0.002; Fig. 11). The reduced investment in reproductive organs thus becomes more pronounced as body size increases and is experienced primarily by female gobies (Fig. 11). In this case, the intensity of infection was a better predictor of parasite impact than just the presence of parasites (Table 6). Gobies infected with a greater mass of copepods experienced a more severe reduction in gonad mass than those with a lesser infection.

Although body condition was not affected by parasitism, the body mass-age relationship indicates that it takes much longer for gobies infected with *P. tortugensis* to reach a given body mass than those not infected. This trend was confirmed by the ANCOVA, which indicated that the exponent of the mass-age relationship (the rate at which mass increased with age) was reduced in infected gobies compared to those without infections (ANCOVA interaction term: $F_{1,64}$ = 5.33 p = 0.024; Fig. 12). Parasite presence and the intensity of infection were roughly equivalent as predictors of parasite impact on growth (Table 6).

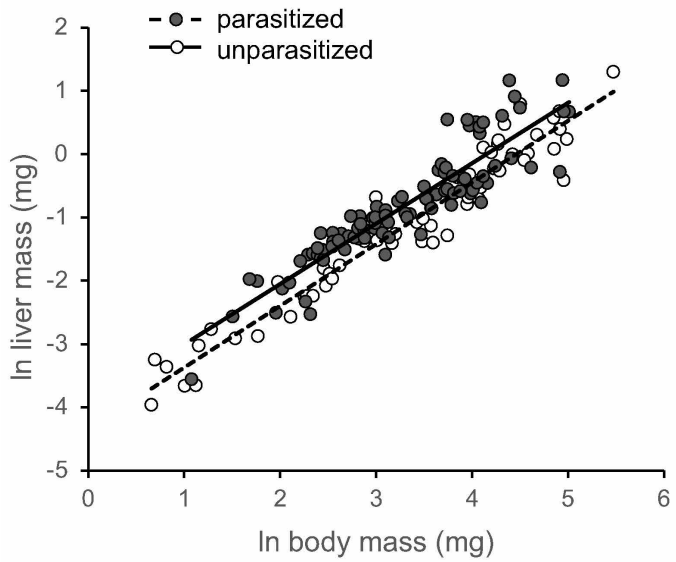

Fig. 10. Liver mass as a function of total body mass in parasitized and unparasitized *C. venezuelae*

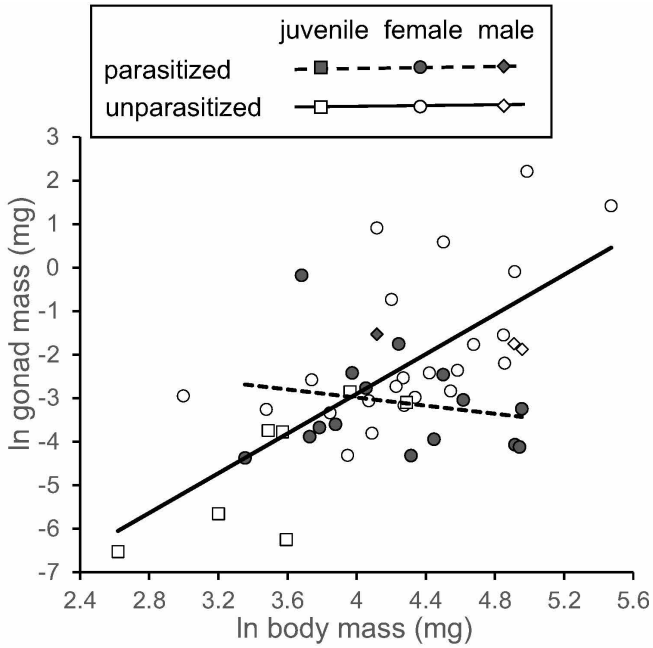

Fig. 11. Gonad mass as a function of total body mass in parasitized and unparasitized *C. venezuelae*.

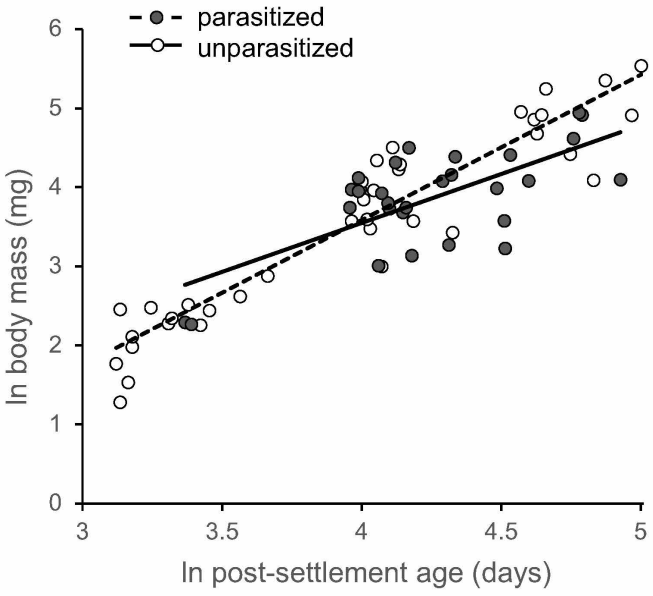

Fig. 12. Total body mass as a function of post-settlement age in *C. venezuelae*.

The host range of *P. tortugensis* in the BVI and Caribbean-wide

P. tortugensis was widely distributed on *Coryphopterus* hosts (*C. glaucofraenum*, *C. venezuelae* and *C. tortugae*) across the BVI and at the few sites searched in the adjoining USVI (infected individuals were detected at 33 of the 52 sites searched; Table 1 and Fig. 2). At the 34 BVI sites where quantitative surveys were done, the prevalence of infection ranged from 1–25 % (mean = 7%, SD = 7%). At the most frequently studied BVI site (Harris Ghut), *P. tortugensis* was present at a consistently high prevalence on *C. venezuelae* for 25 years (mean = 14%, SD = 8%).

Although not definitive, the data suggest the possibility that high prevalence at this site began in the early 1990s, because prevalence was < 5% from 1993–1995 but always > 10% thereafter (Table 2).

Surprisingly, given its ubiquity and relatively high prevalence in the BVI, *P. tortugensis* was either absent or extremely rare on *Coryphopterus* hosts (*C. glaucofraenum*, *C. venezuelae* and *C. tortugae*) at all 16 of other locations searched elsewhere in the Caribbean (Table 3). Despite screening 14 additional goby and blenny species that were potential alternate hosts, we also found no evidence that *P. tortugensis* infects other host species in the BVI (Table 4). None of the 14 additional goby and blenny species sampled was infected by *P. tortugensis*, and this was true both for hosts that were capture and dissected and for those inspected by divers for visible symptoms of infection (Table 4).

DISCUSSION

Individual-level effects of *P. tortugensis*

Like most Chondracanthids, *P. tortugensis* attaches within the opercular cavity of its hosts using clawed antennae (Ho 1971b). The different attachment sites of males and females within the branchial chamber were similar to those reported for congeners *P. banyulensis* and *P. clinii* (Vaney & Conte) infecting the small Mediterranean blennies *Salaria pavo* and *Clinitrachus argentatus* (Risso) respectively (Chabanaud 1951, Rousset and Raibaut 1984). Reasons for the differing attachment sites of males and females are uncertain. The feeding mode of *P. tortugensis* is unknown and, although most chondracanthids feed by scraping host tissues with their mandible, the position of female *P. tortugensis* places their head close to the bulbus arteriosus and adjacent blood vessels of the host. This suggests their attachment sites may be chosen to facilitate feeding on blood (Rousset and Raibaut 1984). The preference of large males for sites adjacent to females may, instead, simply be related to increased mating opportunities.

The extent and type of damage caused by *P. tortugensis* to the gills and branchial chamber of the host goby was comparable to that caused by *P. banyulensis and P. clini* to their blenny hosts (Chabanaud 1951, Rousset and Raibaut 1984). Damage to the host branchial chamber by other gill copepods can lead to severely diminished respiratory efficiency and oxygen uptake (Ojha and Hughes 2001) and reduced host respiratory efficiency seems a likely response to infection by *Pharodes* spp. because both goby and blenny hosts have increased ventilation rates when infected (Rousset and Raibaut 1984, Finley and Forrester 2003). For *Coryphopterus* hosts, a previously unreported consequence of infection with *P. tortugensis*, may be interference with host feeding.

These gobies feed primarily on infaunal invertebrates ingested with mouthfuls of sand. The winnowing mechanism by which prey are separated from sand is not well understood, but involves manipulation of the pharyngeal jaws to create water motion in the buccal cavity, and the expulsion of sand through the opercular cavity (Wainwright and Bellwood 2002). Infected *Coryphopterus* feed at lower rates than uninfected individuals (Forrester et al. 2019), suggesting it would be informative to test whether the presence of *P. tortugensis* alters host diets by disrupting the sorting of food and non-food items.

Because *P. tortugensis* caused extensive damage caused to host respiratory organs and were large relative to their hosts (typically 1-2% of host dry mass), we anticipated that infections would cause a reduction in the energy available for host growth and reproduction. Relatively few other studies have examined allocation to multiple facets of growth and reproduction, making it is difficult to generalize responses among species (review by Johnson et al. 2019). Reduced liver mass and an overall lower body condition are perhaps the most common responses observed in fish with macroparasitic infections (Pennycuick 1971, Gordon and Rau 1982, Lemly and Esch 1984, Collyer and Stockwell 2004, Johnson et al. 2004, Katakura et al. 2004). Unlike several other fish hosts infected with parasitic copepods, *Coryphopterus* hosts showed no reduction in body condition associated with infection (reviewed by Johnson et al. 2019). The mass-age relationship, however, clearly indicated that infected gobies grew in mass more slowly than uninfected ones, as did previous mark-recapture data (Finley and Forrester 2003).

In addition to reduced overall growth, infected *Coryphopterus* had enlarged livers and smaller gonads than uninfected hosts. Whereas the enlargement of the liver was relatively slight and consistent at all body sizes, the reduced investment in reproduction increased with body size and was most severe for larger female *Coryphopterus*. Enlarged livers have been observed in some parasitized fish and are usually associated with a pathological response, stress, or parasites encysting in the liver itself (Takashima et al. 1972, Tierney et al. 1996, Francis 1997, Malek 2001). We found no parasites in the livers of *Coryphopterus* and so can exclude this possibility. A pathological or stress response to initial infection would be expected to be greatest in young fish and decrease with age, as observed when sticklebacks are infected with cestodes (Tierney et al. 1996), and so is not consistent with our data. Consistent liver enlargement may thus reflect prolonged stress leading to fatty degeneration and impaired liver function (Hilton and Dixon 1982, Shul′man and Love 1999). Biochemical analysis of lipid levels in the liver would resolve physiological and metabolic differences in parasitized and unparasitized fish and determine the cause and consequence of differences in liver size.

An additional possible contributor to increased liver size in parasitized individuals is a trade-off in resource allocation to energy storage and reproductive output. Reduced gonad mass is

perhaps the most common reproductive impact of parasitic copepods and is argued to result from general host debility (Kabata 1984). In *Coryphopterus* hosts, reductions in gonad size associated with parasitism were experienced primarily by females. This sex-related bias is partly related to protogynous hermaphroditism; these gobies maturing first as female at around 55 mg (24 mm SL) and then changing sex to male around 120 mg (35 mm SL) (Cole and Shapiro 1990, 1992). It is also partly because very few large *Coryphopterus* > 120 mg are infected with *P. tortugensis*. Effects of parasitism on reproduction in sex changing fish are rarely studied, but these two findings suggest the potential for infected juveniles and small females to allocate energy to lipid storage and growth rather than reproduction as a life-history response to infection. If growing to a larger large size facilitates shedding infection or reduces its impact, deferring female function may increase reproductive value via the dual benefits of improved survival and future male reproductive function (Warner 1988).

In aquaculture settings, where infection intensities can be very high, it is common for the severity of parasite impacts on individual hosts to increase with the burden of infection (reviewed by Johnson et al. 2019). For free-living *Coryphopterus*, we found no evidence that a greater mass of copepods was associated with stronger impacts on growth or liver mass, but decreases in gonad mass were more severe for *Coryphopterus* with high-intensity infections (see also Katakura et al. 2004). We cannot certain why infection intensity affected only reproductive allocation. One possibility is limited variability in infection intensity. Like most macroparasites, *P. tortugensis* is aggregated among individual *Coryphopterus* hosts, but the degree of overdispersion is at the low end of the range observed for other macroparasites of similar mean infection intensity (see Fig. 1 in Poulin 1998), including parasitic crustaceans (Tavares-Dias et al. 2015). This modest degree of overdispersion may be related to the small size of *Coryphopterus* hosts relative to *P. tortugensis*. Limited space within the branchial cavity could simply restrict the maximum number and size of copepods that can infect a host. Consistent with this hypothesis, we rarely observed more than one adult female *P. tortugensis* per opercular cavity, and the same was true for *P. clinii* infecting a similarly sized blenny *C. argentatus* (Chabanaud 1951). In contrast two or three *P. banyulensis* females were routinely observed in each opercular cavity of a larger blenny host (*S. pavo*) (Rousset and Raibaut 1984).

The scope of population- and community-level impacts of *P. tortugensis*

The number and extent of disease outbreaks in marine organisms is argued to be increasing (Lafferty et al. 2004), but most data comes from overt outbreaks and a lack of baseline data makes reliable estimates difficult (Ward and Lafferty 2004). *P. tortugensis* was previously reported on 11

fish species, mostly blennies and gobies in the Western Atlantic (NMNH 2020, WORMS 2020), but these reports come from museum specimens and so lack ecological context. Our field surveys identified what appears to be a persistent outbreak of *P. tortugensis* infections in the BVI that is limited a subset of its known hosts (four *Coryphopterus* species). One of the few baseline surveys in marine fish, also showed that visible symptoms of disease in Dab (*Limanda* Linnaeus) were clustered in space and stable over several years (Stentiford et al. 2009). Further work to assess the spatial and temporal consistency of outbreaks would thus be informative.

Many parasites infect multiple host species and are widely distributed across heterogeneous sea- and landscapes (Johnson et al. 2015). In this setting, just one or a few host species can maintain a high prevalence of infection that allows the parasite to persist in the area, but the reasons why certain hosts and sites act as the primary reservoir for infections are not well understood (Wilber et al. 2020). We cannot explain why the outbreak of *P. tortugensis* was limited to just four of its fifteen known hosts and nor can we explain why the BVI was a hotspot for infections. One possibility, unusual crowding or aggregation of hosts can be eliminated because ecological studies of *Coryphopterus* elsewhere show that the BVI is unremarkable in these respects (e.g., Forrester and Steele 2004). Interestingly, one of our BVI sites (White Bay, Guana Island) was also a site of high prevalence for infections of isopods on French grunts (Welicky and Sikkel 2014) and of monogeneans on surgeon fish (Sikkel et al. 2009). If further work confirms that certain sites are hotspots for multiple sets of host-parasite interactions, then environmental factors, such as climate or pollution, may be the underlying cause (Behringer et al. 2020).

CONCLUSIONS

Coupled with past population-level analyses, this study provides a comprehensive assessment of impacts of *P. tortugensis* on four of its hosts, which appear to be plausibly interrelated from the individual-level to community-level. Like many other copepod parasites, *P. tortugensis* damaged the gills and branchial chamber of *Coryphopterus* hosts, which seems to compromise respiratory function and possibly feeding. This damage appears to slow the growth of hosts and alter their energy allocation to lipid storage and reproduction, providing a rare example of strong impacts on individual hosts in nature. These debilitating impacts on individual *Coryphopterus* credibly explain the main population-level impact of *P. tortugensis*, which is to diminish the gobies' effectiveness as competitors for refuges and so increase their vulnerability to predators. We show that this ecologically significant host-parasite interaction appears to be limit to these hosts in the BVI, even though the parasite is a widespread generalist.

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REFERENCES

Andrews M, Battaglene S, Cobcroft J, Adams M, Noga E, Nowak B. 2010. Host response to the chondracanthid copepod *Chondracanthus goldsmidi*, a gill parasite of the striped trumpeter, *Latris lineata* (Forster), in Tasmania. J Fish Dis **33:**211–220. dio:10.1111/j.1365-2761.2009.01107.x.

Aneesh PT, Kappalli S. 2020. Protandrous Hermaphroditic Reproductive System in the Adult Phases of *Mothocya renardi* (Bleeker, 1857) (Cymothoidae: Isopoda: Crustacea) – Light and Electron Microscopy Study. Zool Stud **59:**61. doi:10.6620/ZS.2020.59-61.

Baldwin C, Weigt L, G Smith D, H Mounts J. 2009. Reconciling Genetic Lineages with Species in Western Atlantic *Coryphopterus* (Teleostei: Gobiidae). Smithsonian Contrib Mar Sci **38**.

Baldwin CC, Robertson DR. 2015. A new, mesophotic *Coryphopterus* goby (Teleostei, Gobiidae) from the southern Caribbean, with comments on relationships and depth distributions within the genus. Zookeys **2015:**123–142. doi:10.3897/zookeys.513.9998.

Behringer DC, Silliman BR, Lafferty KD. 2020. Marine Disease Ecology, 1st ed. Oxford University Press, Oxford, UK.

Boxshall G, Hayes P. 2019. Biodiversity and Taxonomy of the Parasitic Crustacea. *In*: Smit NJ, Bruce NL, Hadfield KA (eds) Parasitic Crustacea**:** State of Knowledge and Future Trends Zoological Monographs. Springer International Publishing, Cham, Switzerland, pp. 73–134. doi:10.1007/978-3-030-17385-2_3

Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. J Parasitol **83:**575–583. doi:10.2307/3284227.

Chabanaud P. 1951. Contribution à la morphologie et à la biologie du Copepode parasite *Diocus clini*. Mem Mus Nat Hist Natur **A29:**239–330.

Cole KS, Shapiro DY. 1992. Gonadal structure and population characteristics of the protogynous goby *Coryphopterus glaucofraenum*. Mar Biol **113:**1–9.

Cole KS, Shapiro DY. 1990. Gonad structure and hermaphroditism in the gobiid genus *Coryphopterus* (Teleostei: Gobiidae). Copeia **1990:**996–1003.

Collyer ML, Stockwell CA. 2004. Experimental evidence for costs of parasitism for a threatened species, White Sands pupfish (*Cyprinodon tularosa*). J Anim Ecol **73:**821–830. doi:10.1111/j.0021-8790.2004.00855.x.

Covello JM, Bird S, Morrison RN, Battaglene SC, Secombes CJ, Nowak BF. 2009. Cloning and expression analysis of three striped trumpeter (*Latris lineata*) pro-inflammatory cytokines, TNF-α, IL-1β and IL-8, in response to infection by the ectoparasitic, *Chondracanthus goldsmidi*. Fish Shellfish Immunol **26:**773–786. doi:10.1016/j.fsi.2009.03.012.

Finley R, Forrester G. 2003. Impact of ectoparasites on the demography of a small reef fish. Mar Ecol Prog Ser **248:**305–309. doi:10.3354/meps248305.

Forrester GE, Chille E, Nickles K, Reed K. 2019. Behavioural mechanisms underlying parasite-mediated competition for refuges in a coral reef fish. Sci Rep **9:**15487. doi:10.1038/s41598-019-52005-y.

Forrester GE, Finley RJ. 2006. Parasitism and a shortage of refuges jointly mediate the Strength of density dependence in a reef fish. Ecology **87:**1110–1115. doi:10.1890/0012-9658(2006)87[1110**:**PAASOR]2.0.CO;2.

Forrester GE, Steele MA. 2004. Predators, prey refuges, and the spatial scaling of density-dependent prey mortality. Ecology **85:**1332–1342. doi:10.1890/03-0184.

Francis MP. 1997. Condition cycles in juvenile *Pagrus auratus*. J Fish Biol **51:**583–600.

Gordon DM, Rau ME. 1982. Possible evidence for mortality induced by the parasite *Apatemon gracilis* in a population of brook sticklebacks (*Culaea inconstans*). Parasitology **84:**41–47.

Hadfield KA. 2019. History of Discovery of Parasitic Crustacea. *In*: Smit NJ, Bruce NL, Hadfield KA (eds) Parasitic Crustacea: State of Knowledge and Future Trends Zoological Monographs. Springer International Publishing, Cham, Switzerland, pp. 7–71. doi:10.1007/978-3-030-17385-2_2.

Hilton JW, Dixon DG. 1982. Effect of increased liver-glycogen and liver weight on liver function in rainbow trout, *Salmo gairdneri* Richardson – Recovery from anesthesia and plasma S-35-sulphobromophthalein clearance. J Fish Dis **5:**185–195.

Ho J-S. 1971a. *Pharodes* Wilson, 1935, a genus of cyclopoid copepods (Pharodidae) parasitic on marine fishes. J Nat Hist **5:**349–359. doi:10.1080/00222937100770261.

Ho J-S. 1971b. Parasitic copepods of the family Chondracanthidae from fishes of eastern North America. Smithsonian Contrib Zool **87:**1–39.

Ho J-S. 1970. Revision of the genera of the Chondracanthidae, a copepod family parasitic on marine fishes. Beaufortia **17:**105–218.

Jakob EM, Marshall SD, Uetz GW. 1996. Estimating fitness**:** a comparison of body condition indices. Oikos **77:**61–67.

Johnson PT, De Roode JC, Fenton A. 2015. Why infectious disease research needs community ecology. Science **349:**1069–1078. doi:10.1126/science.1259504.

Johnson SC, Kabata Z, Nowak BF. 2019. Effects of Parasitic Crustacea on Hosts. *In*: Smit NJ, Bruce NL, Hadfield KA (eds) Parasitic Crustacea: State of Knowledge and Future Trends Zoological Monographs. Springer International Publishing, Cham, Switzerland, pp. 267–329. doi:10.1007/978-3-030-17385-2_6.

Johnson SC, Treasurer JW, Bravo S, Nagasawa K, Kabata Z. 2004. A review of the impact of parasitic copepods on marine aquaculture. Zool Stud **43:**229–243.

Kabata Z. 1984. Diseases caused by metazoans**:** crustaceans. *In*: Kinne O (ed) Diseases of Marine Animals. Biologische Anstalt Helgoland, Hamburg, Germany, pp. 321–399.

Katakura S, Sakurai Y, Yoshida H, Nishimura A, Konishi K, Nishiyama T. 2004. Influence of the parasitic copepod *Haemobaphes diceraus* and *Clavella perfida* on growth and maturity of walleye pollock *Theragra chalcogramma*. Bull Japan Soc Sci Fish **70:**324–332.

Kottarathil HA, Sahadevan AV, Kattamballi R, Kappalli S. 2019. *Norileca indica* (Crustacea: Isopoda, Cymothoidae) Infects *Rastrelliger kanagurta* Along the Malabar Coast of India –

Seasonal Variation in the Prevalence and Aspects of Host-parasite Interactions. Zool Stud **58:**e35. doi:10.6620/ZS.2019.58-35.

Krebs CJ. 1999. Ecological methodology, 2nd ed. Addison Wesley, Menlo Park, California, USA.

Lafferty KD, Porter JW, Ford SE. 2004. Are diseases increasing in the ocean? Ann Rev Ecol Evol Syst **35:**31–54. doi:10.1146/annurev.ecolsys.35.021103.105704.

Lemly AD, Esch GW. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis machrochirus*: ecological implications. J Parasitol **70:**475–492.

Malek M. 2001. Effects of the digenean parasites *Labratrema minimus* and *Cryptocotyle concavum* on the growth parameters of *Pomatoschistus microps* and *P. minutus* from Southwest Wales. Parasitol Res **87:**349–355. doi:10.1007/pl00008591.

NMNH. 2020. National Museum of Natural History: Invertebrate Zoology Collections. Available at https://collections.nmnh.si.edu. (Accessed 6 Feb. 2020).

Ogle DH. 2016. Introductory Fisheries Analyses with R. CRC Press, Boca Raton, Florida, USA.

Ojha J, Hughes GM. 2001. Effect of branchial parasites on the efficiency of the gills of a freshwater catfish, *Wallago attu*. J Zool **255:**125–129. doi:10.1017/S0952836901001170.

Østergaard P, Boxshall GA. 2004. Giant females and dwarf males**:** a comparative study of nuptial organs in female Chondracanthidae (Crustacea: Copepoda). Zool Anz **243:**65–74. doi:10.1016/j.jcz.2004.07.001.

Østergaard P, Boxshall GA, Quicke DL. 2003. Phylogeny within the Chondracanthidae (Poecilostomatoida, Copepoda). Zool Scripta **32:**299–319. doi:10.1046/j.1463-6409.2003.00113.x.

Pennycuick L. 1971. Quantitative effects of three species of parasites on a population of Three-spined sticklebacks, *Gasterosteus aculeatus*. J Zool **165:**143–162.

Petrik-Finley RJM. 2005. The impact of a parasitic gill copepod on the demography of a reef fish host. PhD dissertation. University of Rhode Island, Rhode Island, USA.

Poulin. 1998. Large-scale patterns of host use by parasites of freshwater fishes. Ecol Lett **1:**118–128. doi:10.1046/j.1461-0248.1998.00022.x.

Richards SA. 2005. Testing Ecological Theory Using the Information-Theoretic Approach: Examples and Cautionary Results. Ecology **86:**2805–2814. doi:10.1890/05-0074.

Robertson DR, Van Tassell JL. 2019. Shorefishes - Homepage., Fishes: Greater Caribbean. A guide to shorefishes of the Caribbean and adjacent areas. Version 2.0. Available at: https://biogeodb.stri.si.edu/caribbean/en/pages (Accessed 21 Sept. 2020).

Rousset V, Raibaut A. 1984. Anatomical and functional effects of *Pharodes banyulensis* infections (Copepoda, Poecilostomatoida, Pharodidae) on *Blennius pavo* (Pisces, Teleostei,

Blenniidae) in a french mediterranean pond (Bassin de Thau). Z Parasitenkd **70:**119–130. doi:10.1007/BF00929581.

Sale PF (Ed.). 2002. Coral Reef Fishes**:** Dynamics and Diversity in a Complex Ecosystem, 1st ed. Academic Press, San Diego, CA.

Samhouri JF, Vance RR, Forrester GE, Steele MA. 2009. Musical chairs mortality functions: density-dependent deaths caused by competition for unguarded refuges. Oecologia **160:**257–265. doi:10.1007/s00442-009-1307-z.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods **9:**676–682. doi:10.1038/nmeth.2019.

Shul′man G, Love RM. 1999. The biochemical ecology of marine fishes, Adv Mar Biol. Academic Press, London, UK.

Sikkel PC, Nemeth D, McCammon A, Williams J Ernest H. 2009. Habitat and Species Differences in Prevalence and Intensity of *Neobenedenia Melleni* (Monogenea: Capsalidae) on Sympatric Caribbean Surgeonfishes (Acanthuridae). J Parasitol **95:**63–68. doi:10.1645/GE-1645.1.

Sikkel PC, Welicky RL. 2019. The Ecological Significance of Parasitic Crustaceans. *In*: Smit NJ, Bruce NL, Hadfield KA (eds) Parasitic Crustacea**:** State of Knowledge and Future Trends Zoological Monographs. Springer International Publishing, Cham, Switzerland, pp. 421–477. doi:10.1007/978-3-030-17385-2_10.

Sindermann CJ. 1987. Effects of parasites on fish populations**:** practical considerations. Int J Parasitol **17:**371–382.

Smit NJ, Bruce NL, Hadfield KA. 2019. Introduction to Parasitic Crustacea**:** State of Knowledge and Future Trends. *In*: Smit NJ, Bruce NL, Hadfield KA (eds) Parasitic Crustacea**:** State of Knowledge and Future Trends Zoological Monographs. Springer International Publishing, Cham, Switzerland, pp. 1–6. doi:10.1007/978-3-030-17385-2_1.

Steele MA, Forrester GE. 2002. Early postsettlement predation on three reef fishes: effects on spatial patterns of recruitment. Ecology **83:**1076–1091. doi:10.1890/0012-9658(2002)083[1076:EPPOTR]2.0.CO;2.

Stentiford GD, Bignell JP, Lyons BP, Feist SW. 2009. Site-specific disease profiles in fish and their use in environmental monitoring. Mar Ecol Prog Ser **381:**1–15. doi:10.3354/meps07947.

Takashima F, Hibiya T, Watanabe T, Hara T. 1972. Endocrinological studies on lipid metabolism in rainbow trout - 1. Differences in lipid content of plasma, liver and visceral adipose tissue between sexually immature and mature females. Bull Japan Soc Sci Fish **38:**307–311.

Tavares-Dias M, Dias-Júnior MBF, Florentino AC, Silva LMA, Cunha AC da. 2015. Distribution pattern of crustacean ectoparasites of freshwater fish from Brazil. Rev Bras Parasitol Vet **24:**136–147. doi:10.1590/S1984-29612015036.

Tierney JF, Huntingford FA, Crompton DWT. 1996. Body condition and reproductive status in sticklebacks exposed to a single wave of *Schistocephalus solidus* infection. J Fish Biol **49:**483–493. doi:10.1111/j.1095-8649.1996.tb00043.x.

Timi JT, Poulin R. 2020. Why ignoring parasites in fish ecology is a mistake. Int J Parasitol Special Issue on 'Fish Parasitology' **50:**755–761. doi:10.1016/j.ijpara.2020.04.007.

Vance RR, Steele MA, Forrester GE. 2010. Using an individual-based model to quantify scale transition in demographic rate functions**:** Deaths in a coral reef fish. Ecol Modell **221:**1907–1921. doi:10.1016/j.ecolmodel.2010.04.014.

Victor B. 2008. Redescription of *Coryphopterus tortugae* (Jordan) and a new allied species *Coryphopterus bol* (Perciformes: Gobiidae: Gobiinae) from the tropical western Atlantic Ocean. J Ocean Sci Found **1:**1–19.

Victor BC. 2015. Western Atlantic *Coryphopterus* gobies., Western Atlantic *Coryphopterus* gobies. Available at http://www.coralreeffish.com/gobiidae2adult.html. Accessed 21 September 2020.

Wainwright PC, Bellwood DR. 2002. Ecomorphology of feeding in coral reef fishes. *In*: Sale PF (ed) Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem. Academic Press, London, UK, pp. 33–55.

Ward JR, Lafferty KD. 2004. The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? PLOS Biol **2:**e120. doi:10.1371/journal.pbio.0020120.

Warner RR. 1988. Sex change and the size-advantage model. Trends Ecol Evol **3:**133–136.

Welicky RL, Sikkel PC. 2014. Variation in occurrence of the fish-parasitic cymothoid isopod, *Anilocra haemuli*, infecting French grunt (*Haemulon flavolineatum*) in the north-eastern Caribbean. Mar Freshwater Res **65:**1018–1026. doi:10.1071/MF13306.

Wilber MQ, Johnson PTJ, Briggs CJ. 2020. Disease hotspots or hot species? Infection dynamics in multi-host metacommunities controlled by species identity, not source location. Ecol Lett **23:**1201–1211. doi:10.1111/ele.13518.

WORMS. 2020. World Register of Marine Species (WoRMS). Available at http://www.marinespecies.org. Accessed 16 February 2020.

Zar JH. 1996. Biostatistical analysis, 3rd ed. Prentice Hall, Upper Saddle River, New Jersey, USA.