

Mancae of the parasitic cymothoid isopod, *Anilocra apogonae*: early life history, host-specificity, and effect on growth and survival of preferred young cardinal fishes

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Abstract Juvenile parasitic cymothoid isopods (mancae) can injure or kill fishes, yet few studies have investigated their biology. While the definitive host of the adult cymothoids is usually a single host from a particular fish species, mancae may use so-called optional intermediate hosts before settling on the definitive host. Little, however, is known about these early interactions. The cymothoid isopod, *Anilocra apogonae*, infests the definitive host, *Cheilodipterus quinquelineatus*. This study examined their host preference among potential optional intermediate hosts. Their effect on the growth and mortality of the young of three apogonid fishes, including the definitive host, was investigated. The number of mancae produced per brood was positively correlated with female length. When given a choice of intermediate hosts, significantly more mancae attached to *Apogon trimaculatus* (Apogonidae) than to *Apogon nigrofasciatus*. When presented with *Ap. trimaculatus* and *Pomacentrus amboinensis* (Pomacentridae), mancae only attached to *Ap. trimaculatus* suggesting that mancae may show a taxonomic affiliation with preferred hosts. Mancae fed on all three apogonid species, with *C. quinquelineatus* being fed on earlier than *Ap. trimaculatus* and *Ap. nigrofasciatus*. Mancae feeding frequency, adjusted for fish survival, was lowest on

C. quinquelineatus and highest on *Ap. trimaculatus*. Infested apogonids had reduced growth and increased mortality compared with uninfested fish. *A. apogonae* mancae can use several species of young apogonid fishes as optional intermediate hosts. Via reduced growth and increased mortality, mancae have the potential to negatively influence definitive host populations and also other young species of apogonid fishes.

Keywords Cymothoidae · Parasites · Coral reef fish · Apogonidae · Isopoda · Great Barrier Reef

Introduction

Cymothoid isopods are large ectoparasites that infest a diverse array of tropical and temperate fishes worldwide (Brusca 1981). They are protandrous hermaphrodites (Bullar 1876) which are unable to leave their hosts after becoming females (Bunkley-Williams 1984). These isopods cause varying degrees of harm in their hosts, ranging from minor tissue damage at the site of attachment to differential mortality (Adlard 1989). Adult isopods in each genus tend to be found at a specific attachment site on their hosts. For instance, adult *Ceratothoa*, *Cymothoa*, and *Irona* are commonly found in the buccal cavity, whereas *Nerocila*, *Renocila*, and *Anilocra* adults generally infest the skin (Brusca 1981; see Jones et al. 2007). First instar mancae can either be found free-swimming (Adlard 1989) or attached to a variety of host fishes, with only some of these species serving as hosts of the adult isopods (Brusca 1981).

The studies of the effects of cymothoids mostly consider their effects on adult fish, rather than interactions between young isopods and young fish (but see Bunkley-Williams

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1984; Segal 1987; Adlard and Lester 1995; Papapanagiotou et al. 1999; Šarušić 1999; Williams and Bunkley-Williams 2000; Papapanagiotou and Trilles 2001; Mladineo 2003). Little is known about the host-specificity, feeding frequency, and effect on the host of the first instar free-swimming mancae stage (Brusca 1981; Bunkley-Williams and Williams 1998).

Young fish, because of their relatively small size compared to that of the cymothoids, are likely to be more vulnerable to infestation than are adult fish. Evidence is accumulating that some cymothoid mancae injure and kill larval and juvenile fish of commercially and recreationally important species (see Brusca 1981; Bunkley-Williams and Williams 1998; Papapanagiotou et al. 1999; Šarušić 1999; Horton and Okamura 2001; Papapanagiotou and Trilles 2001; Mladineo 2002, 2003). The high mortality in young fish due to young cymothoids has the potential to be more important than the damage done to adult fish by adult cymothoids (Adlard 1989; Bunkley-Williams and Williams 1998).

The free-swimming (manca) stage usually locates and attaches to hosts, particularly young hosts (Menzies et al. 1955; Kroger and Guthrie 1972; Garrey and Maxwell 1982; Bunkley-Williams 1984; Adlard and Lester 1995; Marks et al. 1996; Colorni et al. 1997; Leonardos and Trilles 2003). In associations involving the definitive, or final, host, isopod growth is correlated with host growth. Mancae are free-swimming stages that are released from the adult female's marsupium; there is no larval stage (Bunkley-Williams and Williams 1998). They are usually immediately infective to suitable fish hosts, upon which they feed on until they molt into a juvenile (or "aegathoid") stage (Brusca 1978a; Adlard and Lester 1995).

The time from the release of mancae from the marsupium to competency for attachment is brief (Sandifer and Kerby 1983; Adlard 1989; Tsai et al. 1999; Mladineo 2003). Mancae that do not find an appropriate host perish within about a week (mancae are unable to feed whilst free-living) (Menzies et al. 1955; Sandifer and Kerby 1983). In this critical period of host location, micropredation (taking a meal and leaving, then infesting other hosts) (Lafferty and Kuris 2002) and typical parasitism (staying attached and feeding intermittently) on so-called optional intermediate hosts prolongs the life of mancae. These are 'optional intermediate hosts' because these other fish species are not a necessary part of the isopods' lifecycle.

The few documented examples of interactions with optional intermediate hosts were ephemeral and eventually resulted in either host death or isopod loss (Segal 1987; Adlard 1989; Bunkley-Williams and Williams 1998; Šarušić 1999). The fate of the optional intermediate host

fish that do survive, however, has not been documented. If the optional intermediate hosts die, the mancae can detach and seek another host. When they attach to the definitive host, they lose their swimming ability and mature as a female (Brusca 1981; Bunkley-Williams and Williams 1998; Tsai et al. 1999; Mladineo 2003).

Whether mancae become micropredators or typical parasites and which species they infest seems partly to depend upon the degree of their host-specificity (Brusca 1981; Adlard 1989). Some species are highly host-specific and even as mancae only have one suitable host (Tsai et al. 1999). More commonly, cymothoids have low host-specificity, and mancae may attach and feed on optional intermediate hosts, even hosts of different families (Kroger and Guthrie 1972; Lindsay and Moran 1976; Brusca 1978a, 1981; Sandifer and Kerby 1983; Bunkley-Williams 1984; Segal 1987; Adlard 1989; Šarušić 1999).

Mancae are blood and perhaps tissue feeders (Segal 1987; Adlard and Lester 1995; Colorni et al. 1997). Feeding of mancae has been reported for a few species such as *L. ovalis* and *Olencira praegustator* (Lindsay and Moran 1976), *Nerocila acuminata* (Segal 1987), and *Anilocra pomacentri* (Adlard 1989). How much and how often mancae feed on hosts is a likely significant determinant of pathogenicity, but this relationship is yet to be observed (Bunkley-Williams and Williams 1998; Papapanagiotou et al. 1999; Šarušić 1999; Williams and Bunkley-Williams 2000; Papapanagiotou and Trilles 2001; Mladineo 2002, 2003).

Anilocra spp. are among the largest cymothoids. They are conspicuously attached to their hosts, often adhering to the skin on the head and on the anterior third of the body. They are generally highly site specific for a given host (Williams and Williams 1981; Williams et al. 1982; Bunkley-Williams 1984; Adlard 1989). *Anilocra apogonae* commonly infests the Indo-Pacific five-lined cardinal fish, *Cheilodipterus quinquelineatus* at Lizard Island, Great Barrier Reef (Östlund-Nilsson et al. 2005). Isopods are permanently attached posterodorsally to the eye of the host. The isopod faces forward, and always occurs as single infestations (Fig. 1). Adult *An. apogonae* have a marked effect on the short-term fitness (swimming speeds, oxygen consumption, hunger levels) (Östlund-Nilsson et al. 2005) of *C. quinquelineatus*. Mancae-host relationships have not been investigated.

To examine the interactions between *An. apogonae* mancae and late-stage larval and juvenile fish, the life history of *An. apogonae* was described; host preference of the mancae was experimentally investigated; and growth and mortality of fish infested with a mancae were compared to uninfested fish for three apogonid species.



Fig. 1 Three *Cheilodipterus quinquelineatus* infested with a size range of *Anilocra apogonae*

Materials and methods

Fish and isopod collection

The study was conducted at Lizard Island Research Station, Great Barrier Reef, Australia (14°40' S, 145°28' E). Collection of all fish (*Apogon trimaculatus*, *Apogon nigrofasciatus*, *C. quinquelineatus* (Apogonidae), and *Pomacentrus amboinensis* (Pomacentridae) was between 1 November and 29 December 2004. Larval fish (except *C. quinquelineatus*) were collected using light traps (Stobutzki and Bellwood 1997) during 9–20 November and 6–18 December. Light traps were moored off the reef, over sand, in front of Lizard Island Research Station. Traps were set out every night and emptied each morning (~0700 h). Larval fish were used because the authors frequently witnessed different species caught in the light traps which were already infested. We also witnessed larval fish infestation by isopods in nature. *Ap. trimaculatus*, *Ap. nigrofasciatus*, and *P. amboinensis* were chosen as study species due to their abundance in light trap catches. They are also closely related (except *P. amboinensis*) to the definitive host, have habitat overlap, and *An. apogonae* are not found on these fish as adults, which suggests that they are unsuitable hosts. All fish collected were immediately placed into holding aquaria with flow-through seawater at ambient temperature and aeration and fed with live *Artemia salina* nauplii and wild plankton.

Juvenile *C. quinquelineatus* were collected by scuba from small reefs and rocky outcrops using localized sprays of clove oil anesthetic (100 ml of clove oil and 400 ml of ethanol; Munday and Wilson 1997). Anesthetized fish were caught with a 1 mm mesh hand net, placed in separate hermetically sealed plastic bags underwater, then transferred to 10 l buckets for transport to the laboratory. Juvenile fish infested by isopods have also been seen in nature, but it was not possible to identify whether or not fish that were caught were previously infested.

To obtain mancae, adult *C. quinquelineatus* infested with ovigerous *An. apogonae* were collected from reefs using clove oil. Gravid female isopods were identifiable by their full, dark marsupium and their protruding posterior which was characteristically pushed away from the body of the host. The term “manca(e)” will refer only to *An. apogonae* unless otherwise noted.

Freshly released mancae were obtained from isopods in one of two ways: (1) mancae were released from the marsupium of the female in small pulses or all at once if pressure was applied to its dorsal surface, or (2) female isopods on stressed adult fish (due to the anesthetic or to handling) expelled mancae (whether fully developed or not) from their marsupium.

Life history of mancae of *An. apogonae*

To estimate isopod brood size and the relationship with female size, the total lengths of ovigerous females collected from adult *C. quinquelineatus* were measured to the nearest 0.01 mm, and the number of larvae in the marsupium of live females was counted. The total lengths (including uropods) of fully developed mancae were measured after expulsion from the marsupium. The behavior involving the release of mancae from the marsupium of adult females was recorded, as was the manca's attack behavior when presented with a fish.

To observe their longevity and behavior, a subgroup of 50–100 newly released mancae were held in a PVC pipe (diameter 12 cm × 25 cm) with 62 μm mesh netting on both ends of the pipe and supplied with recirculating seawater. This prevented mancae from touching the sides of the aquarium and attempting to crawl out. Manca survival was recorded daily. Death was defined as the time when pleopod movement ceased and there was no reaction to tactile stimulus with a glass pipette.

Host preference

Two preference experiments assessed host preference of *An. apogonae* mancae. Mancae were given a choice of two apogonid species, *Ap. nigrofasciatus* and *Ap. trimaculatus* ($n = 8$ trials), and a choice between an apogonid

(*Ap. trimaculatus*) and a pomacentrid (*P. amboinensis*) ($n = 8$ trials). Juvenile *C. quinquelineatus* were not available at the same time as the other larval apogonid fishes and thus were not included in the host preference experiments. Control fish from the same cohort were selected at random from holding tanks and treated in the same way (but without the addition of a manca). One *Ap. nigrofasciatus* (or *P. amboinensis*) and one *Ap. trimaculatus* of similar size was placed in a blue, plastic container (17 cm long, 11.5 cm wide, 6.5 cm deep), with 500 ml of seawater kept at a constant temperature (28–29°C). Blue permitted mancae to be easily seen. No flow-through seawater was provided for the initial infestation to prevent the manca from escaping through the outlet holes. A single, freshly-released manca (a few minutes old) was introduced into a container (using a 5 ml pipette) with the fish and allowed to infest either. Once one was infested, both fish were removed from the blue containers and held in running seawater in separate clear, plastic 200 ml vials with three 2 mm diameter outlet holes to allow flow-through of running seawater.

Effect of manca on young apogonids

Infested *Ap. nigrofasciatus* and *Ap. trimaculatus* from the host preference experiment were kept to examine the effect of a manca on host growth and mortality. As no mancae infested *P. amboinensis* in the above experiment, this third species was not included here. To examine the effect of mancae on young definitive hosts, *C. quinquelineatus* was also included. For this species, one freshly released manca was introduced as above into a clear container containing a juvenile fish. Juveniles of the definitive host were used as no larvae were collected in light traps.

Data collected in all experiments

Attachment: host- and site-specificity

All fish were observed continuously in the three experiments above until the manca attached to a fish. The duration from manca introduction to attachment time, the species of fish that the manca attached to, the site of attachment (posterodorsal to the eye, mouth, ventral surface), and the number of successful infestations in each of the experiments were recorded. As a measure of infestation susceptibility, the time it took for a manca to attach to *Ap. trimaculatus* or *Ap. nigrofasciatus* was compared between species using Analysis of Variance (ANOVA) (STATISTICA 7.0). Data were Log_{10} transformed to satisfy assumptions of normality in General Linear Models. Mean \pm SE is used throughout the paper unless otherwise noted. To test for differences amongst host species and

sites of attachment on those species, Pearson chi-square tests were used to compare: (1) the proportion of the host species that mancae infested and (2) the sites they attached to on the three species.

Feeding

If mancae were feeding on the fish, the day that mancae initiated feeding was recorded. Feeding by mancae was recognized by the darkened caeca filled with host blood. A color change in the manca's carapace in relation to feeding was also noted as a potential indicator of molting. Fish that were exposed to a manca but were not infested were excluded from the experiment and returned to the reef. The time it took for a manca to feed on the three fish species was compared using a Poisson distribution with log link function in STATISTICA 7.0. This analysis was selected as the data had a Poisson distribution. The number of times mancae fed per fish was compared among species using an Analysis of Covariance (ANCOVA), with the number of days a fish was infested by a manca as the covariate. Data were Log_{10} transformed to satisfy the assumptions of linearity and normality in ANCOVA.

Host growth

The effect of a manca on host growth (*Ap. trimaculatus*, *Ap. nigrofasciatus*, and *C. quinquelineatus*) was examined by measuring the total length of the infested fish and comparing the measurements to control fish not exposed to a manca. The effect of a manca on the total length (mm) of fish over time (d) was tested separately for each species using a Repeated Measures Analysis of Variance (RM-ANOVA); for ease of interpretation, mean total fish length (mm) over time measured (5-day intervals) was instead plotted. Infested fish were measured (to the nearest 0.1 mm) three to four times at five-day intervals. Each fish was placed in a narrow glass aquarium (3 cm wide, 6 cm long, 8 cm deep) and measured with a measuring tape fastened onto a thin plate of glass. This plate was used to hold the fish against the glass to restrict movement for accurate measurement.

Host mortality

The presence of a manca on the fish (some lost their manca) and fish mortality was recorded daily. Death for each individual was defined as the time when respiratory movements of the operculae ceased and the fish did not respond to tactile stimulus. The two choice experiments and the *C. quinquelineatus* part of the third experiment were carried out for a total of 33 and 29 days, respectively. To test whether the mortality rate differed between infested

fish and their controls, a Kaplan–Meier survival analysis was used in JMPIN 4. For samples where a manca had fallen off the fish during this experiment (i.e., an incomplete observation), a censor was used. Censors are a statistical tool used to identify complete (uncensored) and incomplete (censored) observations; the default codes are a 1 for censored observations and a zero for uncensored observations (Microsoft 1995). These fish were included only in this analysis.

Results

Life history of mancae of *An. apogonae*

Females (13–18.5 mm in length) had broods of 51–178 mancae with the number of mancae per female positively correlated with female length ($r = 0.84$, $n = 24$, $P < 0.0001$; the equation for this linear relationship is: number of mancae = 18.163 (female length mm)— 178.36). The range of mean manca lengths ($n = 12$) per female ($n = 12$) was 2.54–3 mm and the mean of these mean lengths was 2.94 ± 0.11 mm.

Mancae that were fully developed actively swam immediately after their release from the marsupium and were immediately competent to infest larval apogonids. If no potential hosts were available for infestation after release from the marsupium, mancae swam near the surface and appeared to be phototactic. Swimming activity gradually decreased with time. After 2 days, pleopod movement decreased, and after 3 days, no mancae were able to attach to a host.

Mancae always attacked a fish either laterally or from above the fish. Fish usually increased their finning rate and attempted to swim away when in the presence of the isopod. Some fish tried to eat the manca. This usually resulted in the manca attaching to the mouth of the fish. Even in the presence of a potential fish host, mancae appeared to swim in a random direction with rapid loops (klinokinesis). Occasionally, mancae would sink to the bottom of the vial on their dorsal surface and ceased movement; however this did not lead to an attack of the fish from underneath. Mancae only successfully attached to the skin posterodorsally to the eye, on the mouth, or on the ventral surface of the fish.

Host preference

There was no difference between species in the amount of time it took for a manca to attach to *Ap. trimaculatus* (45.8 ± 11.4 ; 1–534 min) and *Ap. nigrofasciatus* (63.1 ± 33.2 ; 1–710 min). Mancae attached to *Ap. trimaculatus* significantly more often (48) than to *Ap. nigrofasciatus* (25) (Pearson chi-square: $\chi_1^2 = 264.5$,

$n = 73$, $P < 0.001$). When given a choice between *P. amboinensis* and *Ap. trimaculatus*, mancae always attached to *Ap. trimaculatus* (35) (Pearson chi-square: $\chi_1^2 = 612.5$, $n = 35$, $P < 0.0001$).

When initiating attachment, mancae were site specific. Mancae on *Ap. trimaculatus* and *Ap. nigrofasciatus* attached primarily posterodorsally to the eye (59; 20, respectively) (Pearson chi-square: *Ap. trimaculatus* $\chi_1^2 = 1564.7$, $n = 82$, $P < 0.001$; *Ap. nigrofasciatus* $\chi_1^2 = 216$, $n = 24$, $P < 0.001$), as opposed to the mouth (17; 2), which occurred most often when fish tried to eat the manca, or on the ventral surface of the fish (6; 2). In contrast, all mancae, when exposed to *C. quinquelineatus*, attached directly posterodorsally to the eye of the fish (43). Mancae that did not attach to the final position on the fish host at the onset of infestation proceeded to crawl over the surface of the fish until permanently residing posterodorsally to the eye. At first contact with the manca, the fish usually reacted violently by rolling around in circles. However, once the manca was established, the fish appeared to behave normally. The proportion of mancae that left hosts after initial infestation (within 23 days) was not significantly different amongst *Ap. trimaculatus* (11), *Ap. nigrofasciatus* (2) and *C. quinquelineatus* (7).

Feeding

Mancae attached to *C. quinquelineatus* initiated feeding significantly earlier (2 ± 0.46 ; 1–7 days) than did those attached to *Ap. trimaculatus* (4.24 ± 0.24 ; 2–8 days) or *Ap. nigrofasciatus* (4.54 ± 0.63 ; 2–10 days) (Poisson Log Model: $\chi^2 = 0.99$, $n = 82$, Wald = 18.9, $P < 0.0001$). The number of times mancae fed on the three different species, adjusted for duration of fish survival, was significantly different with *C. quinquelineatus* being fed on the least (ANCOVA: $n = 128$, $F_{2,2} = 15.1$, $P < 0.0001$; Fig. 2); the number of times a manca fed increased with the duration of fish survival ($P < 0.0001$), and these slopes did not vary among species.

Host growth

Infested *Ap. trimaculatus* were 9% shorter compared with uninfested fish (Repeated Measures (RM)-ANOVA: $F_{1,74} = 7.14$, $n = 76$, $P < 0.0193$; Fig. 3); fish length increased over time ($P < 0.0242$), and this slope was not affected by parasite presence ($P > 0.05$). Similarly, infested *C. quinquelineatus* were 3% shorter than uninfested fish (RM-ANOVA: $F_{1,23} = 7.14$, $n = 25$, $P < 0.0136$; Fig. 3), total length also increased over time ($P < 0.0001$), and this slope was not affected by parasite presence ($P > 0.70$). Infested *Ap. nigrofasciatus* were 20.1 ± 0.89 mm ($n = 17$) and their uninfested controls were 21.8 ± 0.98 mm. The

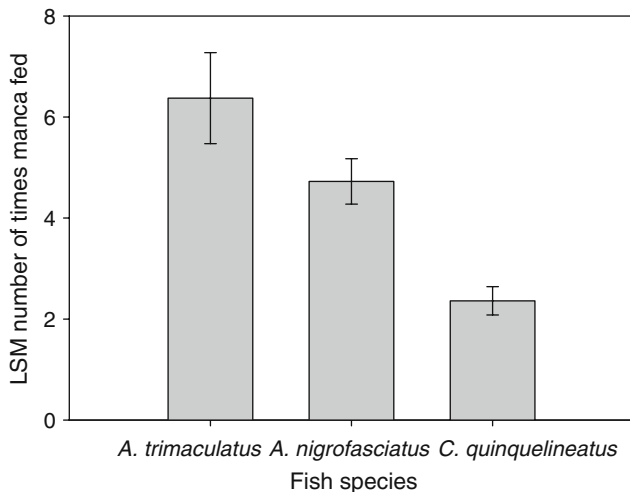


Fig. 2 Least squared means (LSM) (\pm SE) of the number of times an *Anilocra apogonae* manca fed on host fish *Apogon trimaculatus*, *Apogon nigrofasciatus*, and *Cheilodipterus quinquelineatus*, adjusted for fish survival, over 33, 29, and 29 days, respectively

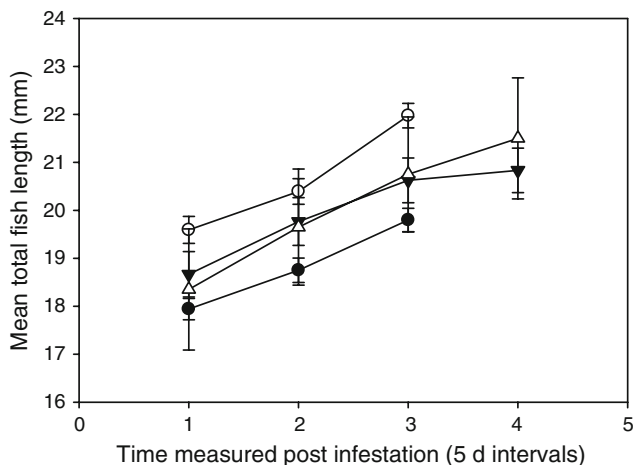


Fig. 3 Mean total lengths (\pm SE) of infested (●) (with an *Anilocra apogonae*) and uninfested (○) *Apogon trimaculatus* (○) and *Cheilodipterus quinquelineatus* (△/▽) sampled 5 d post-infestation and then at 5 days intervals

sample size of infested fish that lived or retained a manca ($n = 2$) over 33 days was too small to allow a statistical comparison.

There was no correlation between the total length of fish and the number of times a manca fed on *Ap. trimaculatus* and *C. quinquelineatus*. The sample size for infested *Ap. nigrofasciatus* that lived or retained a manca long enough to be measured was too small ($n = 2$) to test statistically.

Host mortality

Compared to their respective uninfested controls, infested *Ap. trimaculatus* (Kaplan–Meier survival analysis

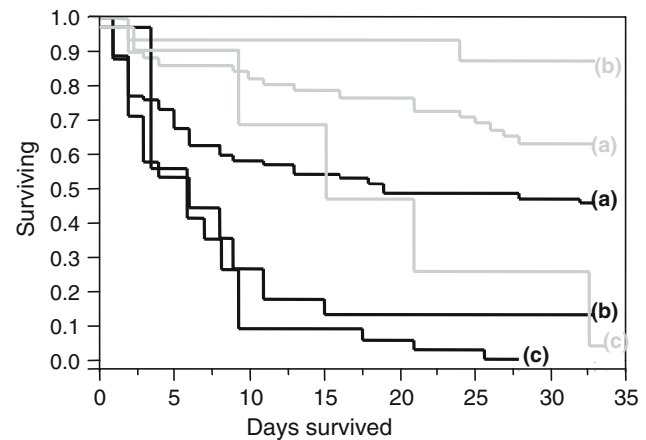


Fig. 4 Proportion of infested (■) and uninfested (□) (a) *Apogon trimaculatus*, (b) *Apogon nigrofasciatus*, and (c) *Cheilodipterus quinquelineatus* surviving over days survived

(KMSA): $\chi_1^2 = 6.8$, $n = 137$, $P = 0.0091$; Fig. 4a), *Ap. nigrofasciatus* (KMSA: $\chi_1^2 = 7.8$, $n = 41$, $P < 0.0001$; Fig. 4b) and *C. quinquelineatus* (KMSA: $\chi_1^2 = 13.6$, $n = 55$, $P < 0.0002$; Fig. 4c) all survived for significantly less time. Of the infested fish, *Ap. trimaculatus* survived the longest, and infested *Ap. nigrofasciatus* lived longer than did infested *C. quinquelineatus* (KMSA: $\chi_2^2 = 14.7$, $n = 164$, $P = 0.0006$). Within the first 48 h of infestation, host mortality ranged from 22% (*Ap. trimaculatus*) to 26.3% (*C. quinquelineatus*).

There was a positive correlation between the duration of *Ap. trimaculatus* survival and the number of times a manca fed on host blood (Multiple Regression: $r = 0.90$, $n = 83$, $P < 0.0001$), adjusted for how long the isopod took to start feeding. The same pattern was observed for infested *Ap. nigrofasciatus* (Multiple Regression: $r = 0.90$, $n = 25$, $P < 0.0001$) and for infested *C. quinquelineatus* (Multiple Regression: $r = 0.67$, $n = 43$, $P < 0.0001$). At the end of the experiment examining the effects of mancae on hosts (29 and 33 days long), mortality varied among the three apogonid species, with the heaviest mortality occurring in infested *Ap. nigrofasciatus* (Pearson chi-square: $\chi_1^2 = 6.063$, $n = 127$, $P < 0.0482$; Fig. 5). To ensure robustness of the results, 95% confidence limits were calculated and parasitized and unparasitized (control) fish did not overlap.

Discussion

Effect of young isopods on host growth and mortality

This study showed that young isopods deleteriously affect young cardinal fish. Newly settled larval *Ap. trimaculatus* and *Ap. nigrofasciatus* and juvenile *C. quinquelineatus*

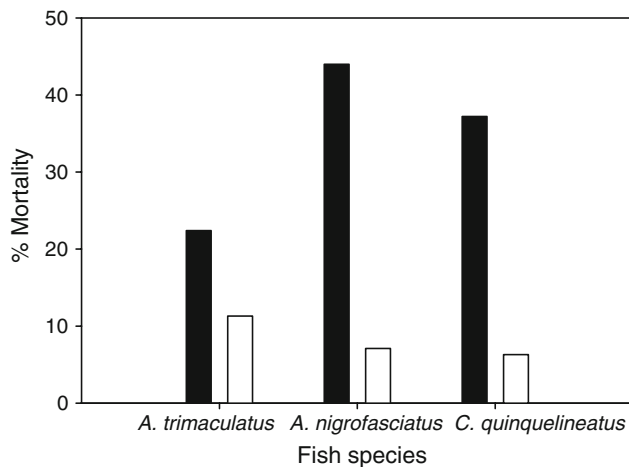


Fig. 5 The mortality (%) of *Apogon trimaculatus*, *Apogon nigrofasciatus*, and *Cheilodipterus quinquelineatus* infested with an *Anilocra apogonae* manca (■) compared with uninfested fish (□)

experimentally infested with mancae of *An. apogonae* had a slower growth rate and a higher mortality rate compared to uninfested fish. Adlard and Lester (1995) also found that fish experimentally infested with *An. pomacentri* mancae had higher mortality rates than uninfested fish. Even though mortality rates were high in fish infested with *An. apogonae* mancae, those that survived infestation lived slightly longer (over 33 days) than did the infested fish (25 days) in Adlard and Lester's (1995) study.

The response of these fish to infestation, however, differed among species. *Ap. trimaculatus* and *Ap. nigrofasciatus* exhibited reduced growth within 15 days, whereas a reduced growth in infested definitive hosts *C. quinquelineatus* was already apparent after only 10 days. Since molting is energetically costly, and *An. apogonae* mancae only molt into the female stage once they attach to their definitive host (Brusca 1978b), mancae do not require as much energy from their temporary hosts as they do from their definitive hosts. This may account for the poorer performance of their natural host. Alternatively, mancae may not be as efficient feeding on temporary hosts as they are on definitive hosts. Other factors that could affect fish response to parasitism, which were not examined here because only the youngest fish available were used, are the size and age of fish. Larger and older juvenile fish may be more physically capable of withstanding infestation, possibly due to relative differences in the size of host and parasite.

Although infested *Ap. trimaculatus* had the lowest mortality rates compared to the other infested apogonids, they also had a slower growth rate. Reduced growth may occur on less common species rarely exposed to a particular parasite (Masuda and Allen 1993). *Apogon trimaculatus* was the least abundant of the three apogonids

and therefore may be less often exposed to this isopod. The initial impact of infestation seems to be the primary reason for death in these larval fish, since most mortality occurred within 48 h of infestation. Those that did survive did not reach the same size as uninfested fish. This pattern was also seen in larval *Dicentrarchus labrax* experimentally infested with mancae of *Ceratothoa oestroides* (Mladineo 2003).

Attachment: host- and site-specificity

Although mancae were not highly host specific among the apogonids tested, they did not attach to the pomacentrids. In contrast, Adlard (1989) found that mancae of *An. pomacentri* attached to six different species of juvenile fish from five families in the laboratory and were also recovered from the comb-tooth blenny and the yellow-striped cardinal fish in nature. However, most of the laboratory infestations on these temporary hosts was short-lived and ultimately resulted either in host death or parasite loss. In this study, the longest host-parasite association was >33 days (16 fish) and the majority of survivors were *Ap. trimaculatus*.

When given a choice between two hosts, a higher proportion of mancae attached to *Ap. trimaculatus* than to *Ap. nigrofasciatus*. There was no difference in the amount of time a manca took to attach to either species, ruling out the possibility that *Ap. trimaculatus* are easier to infest because attachment was easier on those fish. All three of the apogonid species have similar life-histories, habitats, and foraging behaviors. They all aggregate in dark crevices or within branching coral in lagoons during the day, are all found at a depth range of 0–40 m, and all are nocturnal predators (Masuda and Allen 1993). Hence, it is not immediately clear whether *An. apogonae* mancae choose hosts because of taxonomic affiliation or because of ecological preference, as they attached to all three species of apogonids. Since mancae did not attach to any late-stage larval *P. amboinensis*, it appears that there is a strong association of *An. apogonae* and its host family (Bruce 1986). For other cymothoids (e.g., *Livoneca vulgaris*, *Nerocila acuminata*, *An. pomacentri*, and *Telothea henselii*) it appears that choice is based more on isopod ecological preference, or on host availability, rather than on taxonomic affiliation (Brusca 1978a; Segal 1987; Adlard 1989; Taberner et al. 2003).

Since *An. apogonae* mancae successfully attached to *Ap. trimaculatus* and *Ap. nigrofasciatus*, it is concluded that they are optional intermediate hosts for *An. apogonae*. Based on Lafferty and Kuris (2002), *An. apogonae* is a typical parasite of these hosts. It remained attached to the optional intermediate hosts, feeding sporadically, and will ultimately detach, presumably to locate its permanent host.

Attaching to these optional intermediate hosts lengthened the lifespan of the mancae by allowing them to feed (and perhaps molt) in the absence of *C. quinquelineatus*. Although molting of mancae was not specifically observed, a color change of the carapace was noted, which could be indicative of molting or sex change (Brusca 1981). The only time a manca was observed switching hosts (e.g., from *Ap. trimaculatus* to *Ap. nigrofasciatus*; when in the presence of another potential host during the initial attachment experiment) was upon the immediate death of the initial host.

Life history of *An. apogonae*

The range in number of mancae produced by a female *An. apogonae* (51–178) was similar to the brood numbers reported for *An. pomacentri* which also attaches posterodorsally to the eye of their hosts (37–182) (Adlard 1989) and was slightly less than that of isopod species which attach to the host's tongue (62–211) (Menzies et al. 1955; Sievers et al. 1996; Colorni et al. 1997). Factors which may affect the clutch size of isopods are the size of the ovigerous female and the number of broods she produces during her lifespan. The fecundity of many cymothoid species tends to increase with female body size (Menzies et al. 1955; Brusca 1981; Garrey and Maxwell 1982; Adlard 1989; Colorni et al. 1997; Tsai et al. 1999). This was also observed in the present study, where there was a positive correlation between the number of eggs produced in a given female's brood and that female's body length.

Feeding

Mancae attached to all three species of apogonids and fed on the first day of infestation. In contrast, others have suggested that cymothoid mancae do not feed at the onset of infestation because of yolk storage (Menzies et al. 1955; Brusca 1978a; Sandifer and Kerby 1983; Segal 1987). Mancae did not feed as often on host *C. quinquelineatus* blood, as they did when attached to *Ap. trimaculatus* or to *Ap. nigrofasciatus*. One explanation for this is that mancae fed more on these optional intermediate hosts rather than on *C. quinquelineatus*, perhaps because they may have evolved not to over-burden or kill their definitive hosts with whom they have a life-long association, whereas optional intermediate hosts are more expendable (A.M. Kuris personal communication).

There was no association between the number of times a manca fed on a host and host growth. However, the number of times a manca fed on a given host was positively correlated with survival for all three species; this apparent contradiction may merely have been due to the duration of host survival (the longer the host lives, the more the manca feeds). The lack of association between feeding and growth

could be due to the presence (physical damage and stress caused by attachment independent of the consequences of feeding) of the manca alone, causing growth retardation and higher mortality rates. It is unclear whether cymothoid mancae have comparable effects on fish in nature, however. Pathological consequences for fish infested with cymothoid mancae may depend on resources such as food.

In order to accurately quantify isopod effects on host fish populations, the negative impacts on both adult and young fish must be taken into account. This is because small fish, whether they are larval or juvenile, are at a greater risk to micropredation and parasitism than are adults (Segal 1987), since the impact of being infested alone has the ability to kill a young host.

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