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Behavioral and ecological correlates of foureye butterflyfish, *Chaetodon capistratus*, (Perciformes: Chaetodontidae) infected with *Anilocra chaetodontis* (Isopoda: Cymothoidae)

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Abstract: We observed the behavior and ecology of *Chaetodon capistratus* infected and uninfected with the ecto-parasitic isopod *Anilocra chaetodontis* to assess whether there may be parasite induced alterations in host biology, host defenses against infection, and/or pathology related to infection. We also examined habitat related differences in infection rates. Infected fish had higher rates of interaction with conspecifics and spent more time in low flow environments (which might improve transmission of juvenile parasites to new hosts). Butterflyfish without isopods were chased more frequently by damselfishes, fed more, and had larger territories. Time spent near conspecifics, and fish condition and gonadosomatic index did not vary between infected and uninfected fish. These results suggest that foureye butterflyfish behavior is altered by the isopod parasite in order for the isopods to more easily gain mates or transmit offspring to new hosts.

Key words: Caribbean, coral reef, fish, host behavior, parasite, isopod.

Animals infected with parasites often behave differently than uninfected conspecifics (see reviews in Moore 1995, Poulin 1995). These changes in host behavior can be attributed to three potential factors: 1) the parasite causes a change in the host's behavior to increase the parasite's fitness, 2) the host changes its behavior to rid itself of the parasite, or 3) the parasite causes physiological changes in the host that alter host behavior in ways that are not adaptive for the host or the parasite, i.e. the changes are side-effects of the infection pathology (Moore 1995, Poulin 1995). Much research has shown that intermediate host behaviors are altered by parasites to improve parasite transmission to its definitive host (reviews in Holmes and Bethel 1972, Moore 1995, Poulin 1995). There are far fewer studies examining whether parasites alter behavior in definitive hosts (see reviews in Dobson 1988, Poulin 1995).

Anilocra is a genus of marine cymothoid isopods that are ectoparasites of fishes (Williams and Williams 1981). They are protandrous hermaphrodites and internal fertilizers. Juvenile *Anilocra* are able to directly infect another host fish (Williams *et al.* 1982, Williams and Williams 1985, D. Meadows and C. Meadows, pers. obs.), though they may also have non-obligate intermediate hosts (Williams 1984). Once *Anilocra* attach to a host, they lose their ability to swim (Williams and Williams 1989). Because close contact between hosts probably increases the likelihood *Anilocra* will find a mate for themselves or a new host for their young, they provide an excellent system in which to look for parasite-induced changes in definitive host behavior.

Anilocra chaetodontis (Williams and Williams 1981) usually infect the area below the eyes of the foureye butterflyfish, *Chaetodon capistratus* (L.). On average, *A. chaetodontis* infecting a single *C. capistratus* weigh 5% of the host's weight (range 2 - 11%, D. Meadows and C. Meadows, unpublished data). Thus, *A. chaetodontis* may be a relatively serious burden to foureye butterflyfishes. *C. capistratus* are territorial and often form monogamous pairs or social groups (Neudecker and Lobel 1982, Gore 1983), which may allow for easier transmission of *Anilocra* (Read 1990). *C. capistratus* also live in a complex, three-dimensional reef habitat that offers opportunities to vary exposure to strong water motion that may affect movement of juvenile parasites to new hosts.

Our objective was to determine the nature of the relationship when *A. chaetodontis* infects *C. capistratus*. If *A. chaetodontis* alter the butterflyfish's behavior to increase the parasite's fitness, based on the above natural history we predict that *C. capistratus* infected with *A. chaetodontis* will: 1) spend more time with conspecifics in order to increase the opportunity for parasite transmission, 2) have more aggressive interactions with conspecifics as a result of these increased interactions, especially those with other social groups, and 3) spend more time in low flow environments where the transmission of *Anilocra* should be more successful. If *C. capistratus* infected with *A. chaetodontis* were trying to minimize parasite effects, we predict they will: 1) spend less time and have fewer aggressive interactions with conspecifics, and 2) make more attempts to rid themselves of parasites. Specific predictions for changes under the side-effects hypothesis are not possible *a priori*. We also examined whether infection rates varied with habitat as others have found in other ectoparasite systems (Poulin 1995, Sikkel *et al.* 2000).

MATERIALS AND METHODS

The study was conducted on eight reefs off the southwest coast of Puerto Rico, near La Parguera (17°57'N, 67°02'W) in the summer of 2000. We conducted 20 min activity budgets of haphazardly chosen *C. capistratus* both with (N=63) and without (N=42) isopods using SCUBA at depths of 2 to 14 m on four of the reefs. We noted the number of visible isopods per host, and visually estimated the size of the fish (± 5 mm Standard Length, SL) and the isopod (± 5 mm Total Length, TL). In our activity budgets we counted the feeding rate of the fish, the number of chases with conspecifics and heterospecifics, and we measured the time (in seconds) *C. capistratus* spent in low flow environments (particularly under the cover of soft corals or overhangs) and the time spent interacting socially with one or more conspecifics. To assess host attempts to remove parasites, we also counted the frequency and duration of visits to cleaners and the frequency of scraping behavior in which the host would scrape the area just below the eye and behind the mouth on the substrate. This behavior was suggested to be an attempt by the host to rid themselves of *Anilocra* (E. H. Williams and L. B. Williams, pers. comm.). Territory sizes of *C. capistratus* with (N= 24) and without *A. chaetodontis* (N=23) were estimated in separate 20 min observation periods on three reefs in which we mapped the boundaries of the fish's territory. Territory size was then measured to the nearest square meter. Two way ANOVA's were used to analyze the effect of reef and isopod presence on behavior and territory size. The effect of host fish size and isopod size and load on host behavior were analyzed with separate linear regressions. Data transformations were performed as necessary and are noted in the results.

To assess parasite load and effects of parasites on measures of fish health and fecundity we collected 47 fish and their attached isopods using hand spears. The SL of fish, and TL of isopods, were measured to the nearest millimeter. Isopods and fish were also wet weighed (± 0.01 g), as were the gonads of the fish. These data were used to calculate actual isopod load, fish gonadosomatic index (GSI, % of body weight consisting of gonads) as a measure of reproductive effort, and fish condition (weight divided by length cubed and then

TABLE 1

Mean (± 1 s.e.) activity levels, territory size, and health measures for C. capistratus *infected and uninfected with* A. chaetodontis

Behavior	Uninfected	Infected
Time with other *C. capistratus*	245 (48)	141 (33)
Chases with other *C. capistratus*	0.69 (0.3)	2.61 (0.9)
Chases to conspecifics	0.33 (0.19)	1.22 (0.46)
Chases from conspecifics	0.36 (0.23)	1.39 (0.55)
Time in low flow environments	76 (22)	330 (42)
Chased by damselfishes	7.7 (1.0)	2.9 (0.4)
Feeding rate	62.2 (5.9)	37.9 (3.6)
Territory size (m^2)	17.4 (2.7)	14.4 (2.0)
Fish Condition	4.2 (0.1)	4.4 (0.2)
Gonadosomatic index	0.8 (0.3)	0.9 (0.8)

Activity data are from 20 min activity budgets. Chases between conspecific *C. capistratus* are subdivided based on whether the focal fish was the aggressor or recipient in an interaction. For sample sizes see Methods.

multiplied by 100) as a measure of fish health (Anderson and Gutreuter 1983). One-way ANOVA's were used to determine if infected and uninfected fish differed for these variables. Data from the regression of isopod weight on isopod size were used to calculate isopod load for the isopods observed on fish in the behavior study.

Infection rates in different habitats were estimated by visual swimming 6 m wide transects of 40 min duration along eight reefs. We recorded the number of fish of each parasite load on each reef and visually estimated fish SL and parasite TL as before. We also compared infection rates within reefs by depth by having equal length swims at both 4 and 12 m depth on each reef and analyzing with a paired *t*-test.

RESULTS

In our transect study we observed 353 foureye butterflyfishes. Of this number, 233 were uninfected by *A. chaetodontis*, 68 were infected by one isopod, and 52 were infected by two or more isopods. This distribution reflects a higher than expected chance of double isopod infection based on a random distribution of isopods (X^2 = 80.2, p<0.001).

Neither the length or weight of the isopods, nor the reef site, nor the length of the host fish had any relationship to host fish behavior, so these data are not discussed further. However, the presence or absence of isopods was related to some aspects of behavior (Table 1). Butterflyfish with isopods did not differ in the amount of time spent with conspecifics (ANOVA: df = 1-97, F = 3.4, p = 0.07) but did have more aggressive interactions with other foureyes' (ANOVA: df = 1-97, F = 4.2, p = 0.049). Infected fish spent significantly more time under cover in low flow environments (ANOVA: df = 1-97, F = 33.6, p<0.001, log transformed) and had fewer chases from damselfish (ANOVA: df = 1-97, F = 25.7, p< 0.001, log transformed). Fish infected with *A. chaetodontis* also fed less (ANOVA: df = 1-97, F = 10.5, p = 0.002, log transformed) and had smaller territories (ANOVA: df = 1 - 41, F = 5.2, p = 0.03, log transformed) than those without isopods. Scraping and cleaning behavior were too rare to analyze statistically, but did not appear to differ with infection. Neither fish condition (ANOVA: df = 1-45, F = 0.6, p = 0.4) nor Gonadosomatic index (ANOVA: df = 1-46, F = 0.6, p = 0.4) varied with isopod infection. There were more isopod infections in deeper waters than shallow (deep, $\bar{x}$ = 41.8%, shallow, $\bar{x}$ = 33.9%, *t* = 2.7, p = 0.03).

DISCUSSION

The fact that *A. chaetodontis* occurs more frequently in pairs or groups on *C. capistratus* than expected based on a random distribution of isopods supports the parasite-manipulating-host hypothesis or the hypothesis that infected fish are weaker and/or more susceptible to infection. The hypothesis that *A. chaetodontis* alters *C. capistratus* behavior to find a mate or host for their young is further supported by the increased time of infected *C. capistratus* in low flow environments and the greater rates of interaction of infected *C. capistratus* with other foureye butterflyfish. If chases between conspecific butterflyfish are categorized by aggressor and whether each participant is infected with *Anilocra* or not, most interactions occur between infected individuals, whether they were the focal animal or not (Table 1). Uninfected fish rarely chased infected fish. Thus the probability of *Anilocra* transmitting offspring or obtaining mates is likely increased by altering host behavior.

Alternatively, the decrease in feeding and territory size, and the increase in time spent under cover by infected fish could be results of the pathology of infection or could actually be causes of the infection (Poulin 1995). The lower rates of feeding and aggressive interactions with damselfishes, and smaller territory size, in infected fish are probably explained by the increased time under overhangs and beneath soft corals, where interactions with damselfishes and feeding opportunities would be less frequent. However, the combination of increased rates of interaction with certain conspecifics but decreased rates with damselfishes in infected hosts is unlikely the result of typical pathological effects of infection, such as lethargy, and suggests that the increase in interactions with conspecifics is a parasite adaptation. The low rates of interaction of uninfected hosts with infected hosts may be a mechanism to avoid infection. The lack of a difference in time spent with conspecifics suggests this aspect of behavior may not be enough to insure a parasite adaptation. Releasing young in low flow environments may also increase chances of their finding suitable (facultative) intermediate hosts, though this was not studied, and it is unclear what the rate or causation of using intermediate hosts is in this species.

The higher rates of infection in deeper water could result from the lower water velocities and increased parasite transmission or could be the result of host movement to that habitat. The lack of evidence for an effect of the parasite on relative gonad size should mean that the parasite does not destabilize host population dynamics, as suggested by Dobson (1988).

A more definitive determination of the effect of infection with *A. chaetodontis* on foureye butterflyfish ecology and behavior awaits future studies that experimentally infect hosts and that examine the fitness implications of the infection for host and parasite (Poulin 1995). Williams *et al.* (1982) successfully transferred *A. chromis* from brown chromis (*Chromis multilineatus*) to blue chromis (*Chromis cyaneus*) and uninfected brown chromis. They found that experimentally infected brown chromis did not react to the *Anilocra*, while the blue chromis initially reacted with vigorous forward and backward swimming. No long term monitoring of fitness effects occurred.

If the infection of *C. capistratus* by *A. chaetodontis* is a parasite adaptation it would join a small list of other species in which a parasite is found to alter the behavior of its definitive host. For example, Poulin *et al.* (1991) found that a copepod parasite altered the behavior of its fish host to increase the likelihood that the host would be colonized by potential conspecific copepod mates. Similarly, Poinar (1991) and Maeyama *et al.* (1994) found that some mermithid roundworms and hairworms get their insect host to enter water where these parasites can then mate or lay eggs. These effects could be widespread in *Anilocra* and other similar crustacean parasites. *Anilocra* itself includes at least 10 species in the Caribbean that infect at least 25 fish species (Williams and Williams 1998), many ecologically and commercially important. Further research on these parasite-host systems should thus prove fruitful for both basic and applied researchers.

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RESUMEN

Se observó el comportamiento y ecología del pez mariposa de cuatro ojos, *Chaetodon capistratus*, con respecto a la incidencia de infestación con isópodos ectoparásitos (*Anilocra chaetodontis*), para probar si la presencia del parásito altera la biología de su hospedero, comportamiento de defensa de éste contra la infestación y patología relacionada con la misma. También se analizaron diferencias en las tasas de infestación con respecto al tipo de hábitat. Individuos afectados por el parásito presentan tasas más altas de interacción interespecífica y permanecen más tiempo en ambientes de baja hidrodinámica (lo que favorecería la transmisión de parásitos jóvenes a nuevos hospederos). Comparativamente con peces infestados, individuos libres de isópodos parásitos fueron hostigados más frecuentemente por pomacéntridos, se alimentaron más y presentan territorios más grandes. El tiempo de permanencia cerca de conespecíficos, la condición física e índice gonadosomático no variaron entre individuos libres del parásito e individuos infestados. Los resultados sugieren que el comportamiento de *C. capistratus* es alterado por *A. chaetodontis* cuando éste lo parasita, a fin de facilitar al isópodo la obtención de pareja o bien, facilitar la transmisión de su descendencia a nuevos hospederos.

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