FIELD EXPERIMENTAL INFESTATIONS OF ANILOCRA CHROMIS WILLIAMS AND WILLIAMS
(ISOPODA: CYMOPTHIDAE) ON CHROMIS SPP. (PERCIFORMES: POMACENTRIDAE): A
NEW METHOD FOR STUDYING FISH-PARASITE RELATIONSHIPS.

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ABSTRACT: Populations of Anilocra chroma Williams and Williams in the north-eastern and north-western West Indies are parasitic on Chromis multilineatus (Guichenot) and C. cyaneus (Poey), respectively; although both species of fishes occur sympatrically throughout the Caribbean. In St. Croix, U. S. Virgin Islands, where C. multilineatus is naturally infested and C. cyaneus is not, adult females of A. chroma were collected from C. multilineatus and transferred to uninfested C. multilineatus and C. cyaneus. Experimentally transferred A. chroma were significantly less able to survive on C. cyaneus. This population of C. multilineatus seems behaviorally predisposed to the presence of the isopod, A. chroma.
INTRODUCTION

External isopods are among the largest and are the most conspicuous parasites of marine fishes. In the West Indies 12 species of external isopods in the genera Anilocra and Renocila infest many of the most abundant species of coral reef fishes (Williams and Williams, 1977, 1980, 1981). Two of these species, A. holocentra Williams and Williams and A. myriprista Williams and Williams, possess allopatric distributions on holocentrid hosts; squirrelfish, Holocentrus ascensionis (Osbeck), and blackbar soldierfish, Myripristis jacobus Cuvier, respectively. Geographically wide ranging populations of A. acanthura Williams and Williams are allopatrically distributed on 2 closely related hosts; doctorfish, Acanthura chirurgus (Bloch) and ocean surgeonfish, A. bahianus Castelnau. Anilocra chroma Williams and Williams infest brown chromis, Chromis multilineatus (Guichenot) in Puerto Rico, Mona Island, and the U. S. and British Virgin Islands (loc. cit.) (Fig. 1.) A morphologically indistinguishable isopod infests blue chromis, C. cyaneus (Poey), in the Bahamas, south Florida, and the Dominican Republic. Both species of fish occur in all of these areas. We employed a technique developed during aquarium studies of isopods (Williams and Williams, 1978) and the unique facilities of the Hydrolab (NUIS-1) Underwater Habitat to examine why Anilocra chroma did not occur on both species of fishes at the same locality.
MATERIALS AND METHODS

Saturation diving with 4 "aquanauts" was employed from the National Under
water Laboratory System (NULS-1) or Hydrolab Undersea Habitat, at Salt River
Submarine Cayon, St. Croix, U. S. Virgin Islands. Chromis were collected with
handnets and the fish anesthetized quinaldine at the depth of the Underwater
Habitat (14.2 m.), thus allowing manipulations at the ambient pressure of the
host's environment in the underwater laboratory. Chromis not infested with isopods
were collected from an area immediately adjacent to the habitat characterized by
"coral-heads" and defined by steep reef faces and open areas of sand. Chromis
 multilineatus infested with gravid, female Anilocra chroma were collected along
the west wall of the Salt River Submarine Cayon 100 to 200 m from the Habitat,
and also at Habitat depth. Fishes were transported to and held at the Habitat
in individual plastic bags within mesh dive bags, suspended underwater. One
non-infested C. multilineatus or C. cyaneus and 1 C. multilineatus infested with
a gravid, female A. chroma were placed in a shallow, plastic aquarium within the
lockout chamber of Hydrolab. The 2 fish were selected to be of approximately
equal (2 mm or less difference) in standard length. The non-infested chromis
was tagged for individual recognition by injecting acrylic paint under several
scales (Thresher and Gronell, 1978). The isopod was mechanically removed from
the infested chromis, and placed in an identical position on the non-infested
chromis. The artificially infested chromis was placed in a plastic bag filled
with seawater and suspended underwater in a mesh diving bag. The experimentally
infested chromis were returned to the study area immediately adjacent to the
habitat and were individually released in the exact location in which they were
originally captured. No chromis was held for more than 2 hours before the
isopods were transferred. Once infested, chromis were held no less than 45
minutes and no more than 1 1/2 hours. Anilocra chroma were transferred to 22
Chromis multilineatus and 22 C. cyaneus, which were haphazardly collected and released from day 1 through day 5 of the experiment. Experimentally infested hosts were censused each day, as often as water visability, and collection and transfer work, permitted. Position of the study area adjacent to the Habitat and at Habitat depth permitted the senior author 12 hours of observation during 1 diurnal activity period of the hosts. A chi-square test was used to determine if the 2 Chromis spp. differed significantly in retaining experimentally transferred isopods. One dive team (R. E. Waldner and J. J. Kimmel) collected uninfested chromis and released experimentally infested chromis in the study area. The second dive team (L. B. and E. H. Williams) collected infested C. multilineatus and conducted the experimental transfers.
RESULTS

Of the tagged chromis, 14 Chromis multilineatus and 14 C. cyaneus were identified in the field at the close of day 7 of the experiment. More than half of the identifiable C. cyaneus lost A. chroma within 24 hours, and all A. chroma on C. cyaneus were lost in a little more than 2 days (Fig. 2). Only 3 of the identifiable C. multilineatus lost A. chroma and these were lost prior to the first observation after release, possibly indicating improper transfer technique. No. C. multilineatus observed in the field with an experimentally transferred A. chroma ever lost the isopod during the 2-6 days of post-transfer observations. At least 1 tagged C. multilineatus with an experimentally transferred A. chroma attached was observed 22 days after the termination of the experiment, during a follow-up visit to the study area. Others probably survived but could not be confirmed due to the extremely brief observation period, and because scars on many C. multilineatus apparently associated with tagging no longer possessed acrylic paint, and therefore could not positively be interpreted as experimental hosts. Anilocra chroma transferred from C. multilineatus were significantly \( p < 0.05, \chi^2 \) less able to survive on C. cyaneus than on C. multilineatus.
DISCUSSION

Gravid female Anilocra chroma were chosen as experimental animals because they are not capable of swimming from host to host, do not feed, and do not naturally initiate infestations. Gravid females dislodged from hosts in the field or aquaria sink directly to the bottom. They may survive for hours off the host in aquaria, but are usually immediately eaten by fishes in the field. The brood pouch in gravid females is enlarged with reproductive products displacing digestive and other internal organs. The juvenile free swimming stages infest fishes, develop into males, and eventually develop into females (protandrous hermaphrodites).

Female A. chroma must adhere to the fish to survive. Different host will not differ in suitability of ingested materials for this non-feeding stage. Success of the transferred A. chroma is merely a matter of the isopod being able to remain attached to the host. The dactyls (hooks) on the pereopods (legs) of A. chroma from C. multilineatus and C. cyaneus are indistinguishable. The external morphologies of C. multilineatus and C. cyaneus are all but identical. Thus there is no obvious morphological factor which accounts for the observed results.

Specimens of C. multilineatus may obtain a greater standard length than C. cyaneus; however, the standard length of host/total length of isopod relationships are comparable between the 2 species of Chromis (Fig. 3). Therefore, host parasite size relationships should be preserved by the method of transfer, and should not affect the outcomes of transfers.

Removal of a gravid female Anilocra chroma and placement of the isopod on a previously uninfested host is a completely unnatural occurrence for both the isopod and the host. Therefore, no host defense mechanism should exist against a type of infestation which never occurs. Because feeding of the isopod is not
a factor, and the substrates provided by both species of experimental hosts are essentially identical, _A. chroma_ from _C. multilineatus_ should survive equally well when transferred to _C. multilineatus_ or _C. cyaneus_. Surprisingly, no _A. chroma_ on _C. cyaneus_ survived the experiment, while the majority on _C. multilineatus_ survived. Unquantified observations during transfers and immediately following release of newly infested chromis may suggest a possible solution. The level of body movements increased and became more erratic in _C. cyaneus_ exposed to _A. chroma_ than in _C. multilineatus_ so exposed. We suggest that individuals in the population of _C. multilineatus_ studied are behaviorally predisposed to parasitism by _A. chroma_. _Chromis cyaneus_ probably react to the irritation caused by _A. chroma_ until the isopod is eventually dislodged, while _C. multilineatus_ do not. Confirmation of this suggestion must await challenge of members of this population of _C. multilineatus_ with isopods obtained from _C. cyaneus_.

We have found the conspicuousness and abundance of _Anilocra_ spp. in the West Indies to be of value in passive studies of host-parasite relationships in the field. Hochburg and Ellis (1972) employed these isopods as biological tags. Our ability to transfer isopods to a number of different hosts in aquaria (Williams and Williams, unpubl. data) and the results of our experiment, indicate artificial infestation of hosts with _Anilocra_ spp. has great potential for examination of fish-parasite relationships.
ACKNOWLEDGEMENTS

Thanks are expressed to Dr. Thomas E. Bowman, Smithsonian Institution, and Dr. Douglas Y. Shapiro, Department of Marine Sciences, for review of the manuscript; Walter F. Hendrick, Dallas and Chryl Durrance, Edwin Levine, David and Sylvia Shasky, for surface scientific and support diver assistance; Barry B. Walden, Dr. William and Joann Schane, Rod Catanach, Joseph Landsteiner, Dr. Dennis Hubbard, and Dr. Robert F. Dill, for our training and support in Hydrolab. Support was provided by the Manned Undersea Science and Technology Office, Office of Ocean Engineering, National Oceanic and Atmospheric Administration, United State Department of Commerce. (Mission 79-4), through West Indies Laboratory, Fairleigh Dickinson University, St. Croix, U. S. Virgin Islands.
Fig. 1. Brown chromis, *Chromis multilineatus* (Guichenot) (10 cm in total length) infested with a female of *Anilocra chroma* Williams and Williams.
Fig. 2. Cumulative number of blue chromis, *Chromis cyaneus* (Poey) (O) and brown chromis, *C. multilineatus* (Guichenot) (O) which lost isopods after experimental infestation with *Anilocra chroma* Williams and Williams and release in the field.
Fig. 3. Length relationships of the isopod *Anilocra chroma* Williams and Williams and its hosts, *Chromis multilineatus* (Guichenot) (•), and *Chromis cyaneus* (Poey) (X).