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FIELD EXPERIMENTAL INFESTATIONS OF ANILOCRA CHROMIS WILLIAMS AND WILLIAMS (ISOPODA: CYMOTHOIDAE) 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 northeastern and northwestern 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 behaviorly 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 (NULS-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 Underwater 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 anesthethic quinaldine at the depth of the Underwater Habitat (14.2 m.), thus allowing manipulations at the ambient pressure of the hosts' environment in the underwater laboratoy. 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 survied 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 ($p < 0.05$, χ^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 mater 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 factorwhich 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 behaviorly 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.

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Fig. 1. Brown chromis, Chromis multilineatus (Guichenot) (10 cm in total length) infested with a female of Anilocra chroma Williams and Williams.

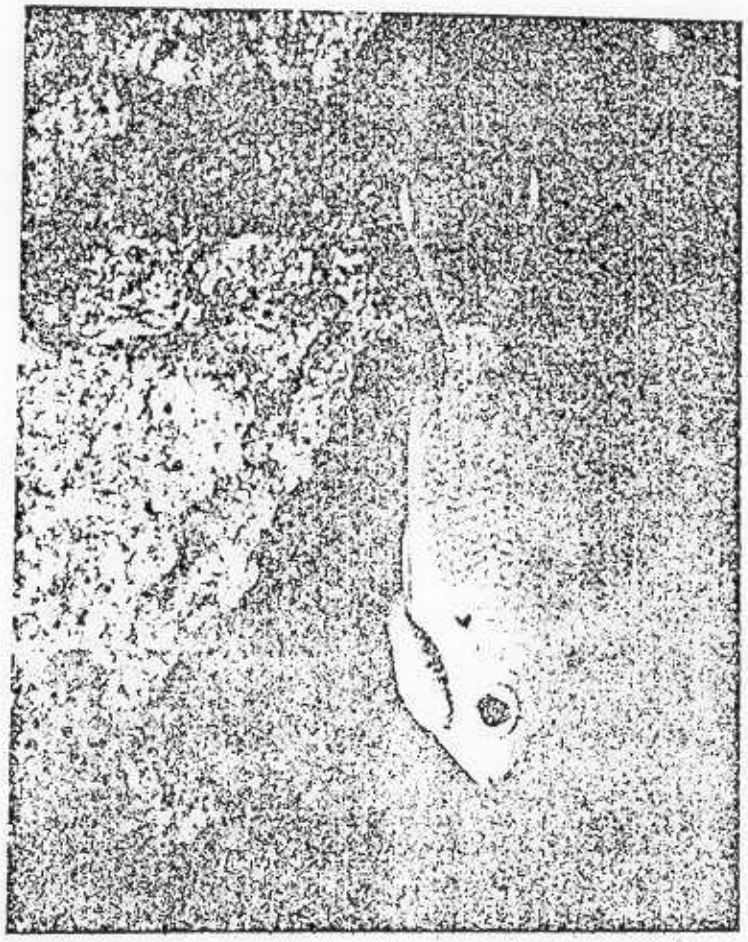


Fig. 2. Cumulative number of blue chromis, Chromis cyaneus (Poey) (0) and brown chromis, C. multilineatus (Guichenot) (0) which lost isopods after experimental infestation with Anilocra chroma Williams and Williams and release in the field.

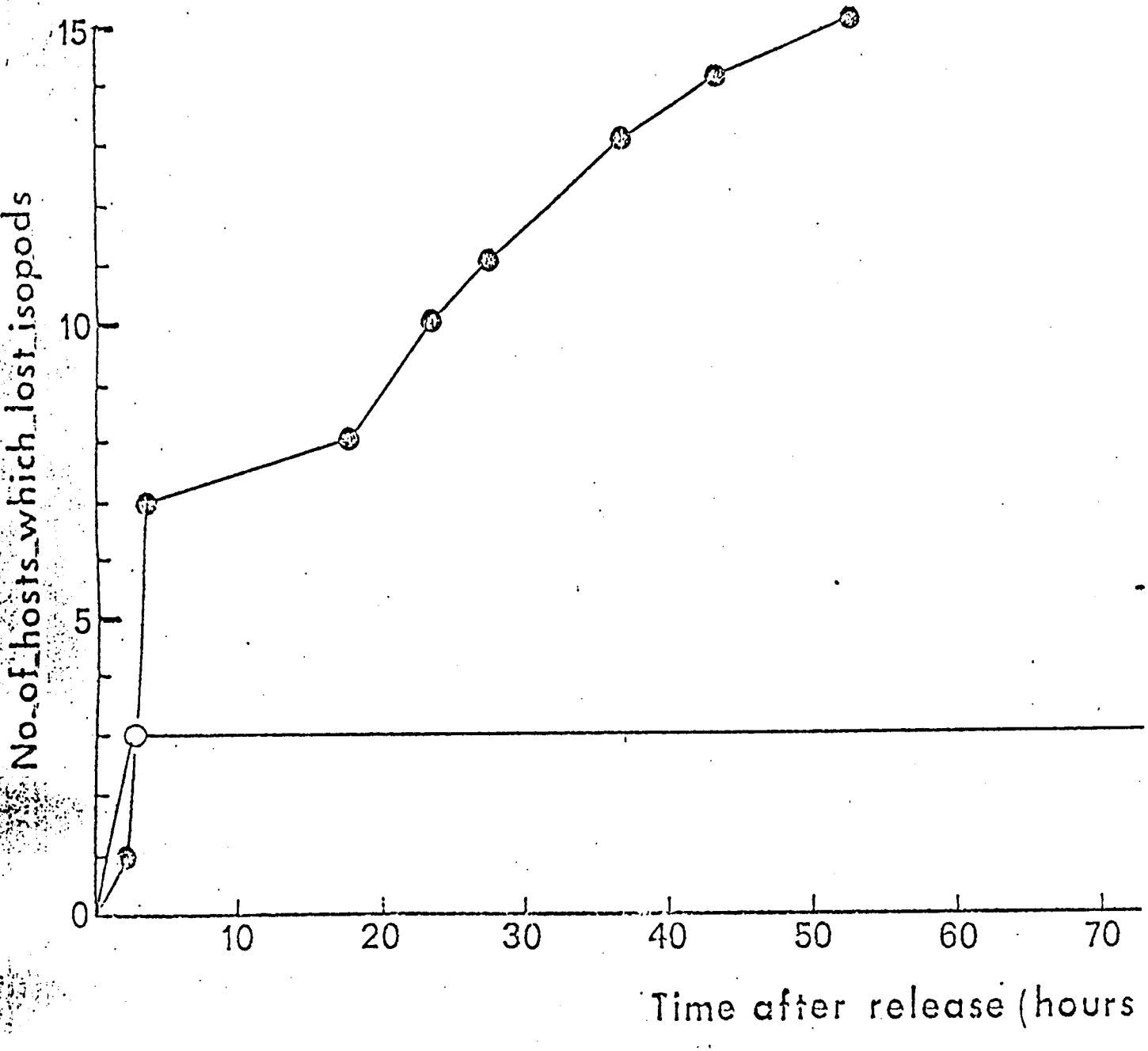
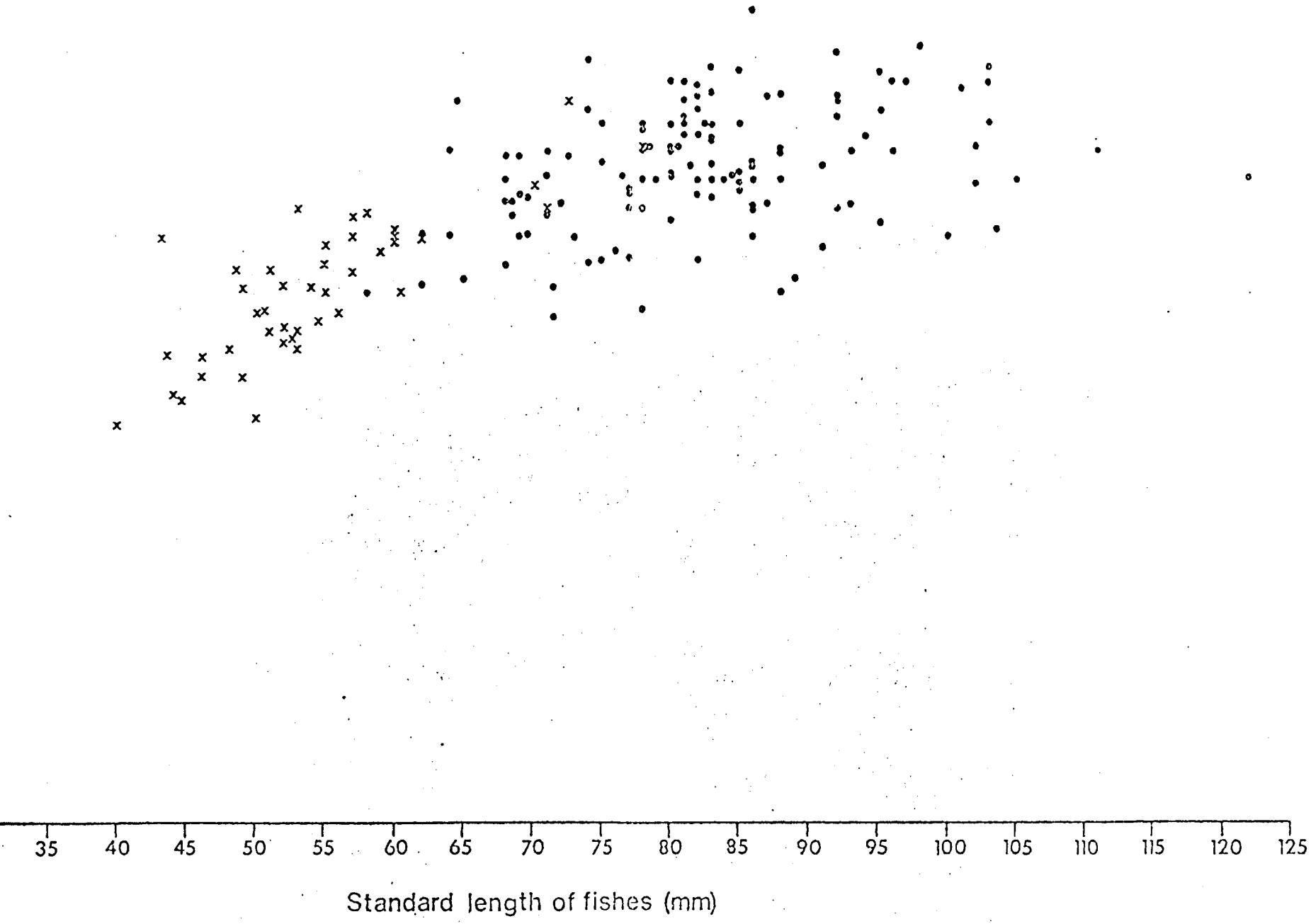

No_of_hosts_which_lost_isopods
15
10
5
0
10
20
30
40
50
60
70
Time after release (hours

Fig. 3. Length relationships of the isopod Anilocra chroma Williams and Williams and its hosts, Chromis multilineatus (Guichenot) (●), and Chromis cyaneus (Poey) (X).


35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
Standard length of fishes (mm)