The Micro-ecology of Three Species of Monogenetic Trematodes of Fishes from the Beaufort–Cape Hatteras Area[1]

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ABSTRACT: Three species of fishes, *Urophycis regius*, *Stenotomus chrysops*, and *Orthopristis chrysopterus* were found to be parasitized by *Diclidophora maccallumi*, *Microcotyle stenotomi* and *Pseudotagia cupida*, respectively. A fourth species of fish, *Peprilus triacanthus*, was not found to be parasitized. The branchial baskets of each species of fish were divided into arbitrary regions and the number of parasites in each region was determined. Site specificity was determined by application of Chi-square tests to the data. *Diclidophora maccallumi*, the only parasite to occur in sufficient number to be tested, showed site specificity. The specific sites of attachments were correlated with the mechanisms of branchial irrigation, and it was suggested that indicated site specificity may be the result of the force and direction of the gill ventilating current.

Early workers in the field of parasitology noticed that some parasites have a higher affinity, or a specificity, for certain parts or regions of the body than others. Workers such as Cerfontaine (1896, 1898) and Gröben (1940), studying the monogenean genera *Diclidophora* and *Dactylogyrus* respectively, found that members of these genera were consistently found on certain areas of the gills. Frankland (1955) studied *Dactylocotyle denticulata* (Olsson, 1876) Yamaguti, 1963 and confirmed Cerfontaine's findings. She further suggested that young specimens of *D. denticulata* are capable of limited movement on the gills, but that this ability decreases with the age of the parasite. Llewellyn (1956) found that the parasites of seven of eleven species of fishes exhibited a site specificity for particular gill arches. He suggested that the upstream position of the diclidophorid posthaptor and the asymmetry of the posthaptor of the diclidophorid *Anthocotyle merlucci* van Beneden et Hesse, 1863, were adaptations to reduce the resistance of these parasites to the gill ventilating currents. Later works by Llewellyn and Owen (1960) on *Discocotyle sagittata*, Owen (1963) on *Diplozoon paradoxum* and Slinn (1963) on *D. sagittata* supported Llewellyn's 1956 findings. Akazaki (1965) working on *Heteraxine heterocerca*, Wiles (1968) working on *D. paradoxum* and Ktari (1969) working with *Microcotyle salpae* further defined these specific areas of attachment by dividing each gill arch into several arbitrary regions. The parasite's position was then indicated with respect to the assigned regions.

The purpose of this study was to further investigate the distribution of monogenean parasites on the gills of their hosts.

Methods and Materials

Host specimens *Urophycis regius* (Walbaum) (Gadidae), *Stenotomus chrysops* (Linnaeus) (Sparidae), *Orthopristis chrysopterus* (Linnaeus) (Pomadasyidae) and *Peprilus triacanthus* (Peck) (Stomateidae) were collected on the continental shelf between Cape Hatteras and Beaufort, North Carolina from November 10–13, 1969 aboard the R/V EASTWARD (Duke University, N. C.). Ten 30-min otter tows were made with a 16-foot try-net (Table 1). Host specimens were identified on board by Dr. J. A. Musick of the Ichthyology Department of the Virginia Institute of Marine Science (VIMS). After fork length measurements of each fish were taken the gills were removed, wrapped in individual gauze packages, and preserved in a solution of 70% ethanol plus 5% glycerol.

In the laboratory the gills were examined for monogeneids with a stereo-microscope and the exact location of the trematodes recorded before removal for identification. A few selected parasites were photographed *in situ*. To indi-

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Table 1. Stations of the R/V EASTWARD at which fishes were taken.

Station No. DUML	Trawl station location Long. (N)	Lat. (W)	Depth in meters	Specimens taken	No.
13245	34°30′	76°44′	21	*P. triacanthus*	10
13276	35°23′	74°55′	100	*U. regius*	13
13279	35°26′	75°03′	30	*U. regius*	3
13282	35°26′	75°20′	18	*U. regius*	8
13285	35°06′	75°17′	53	*U. regius*	1
13290	34°57′	75°19′	182	*U. regius*	10
13293	35°03′	75°23′	51	*U. regius*	1
13300	34°51′	75°49′	30	*S. chrysops*	14
13307	34°26′	75°30′	30	*O. chrysopterus*	10
				U. regius	3
13309	34°27′	76°21′	26	*U. regius*	3

cate the positions of the parasites it was decided to use arbitrary divisions of the gill arches adopted by Wiles 1968 (Fig. 1). Gill arches were numbered from 1–4 anteroposteriorly. Each arch was divided into three equal sections, dorsal, middle and ventral, whereas each holobranch was subdivided into medial and lateral hemibranchs. The surfaces of the hemibranchs were next designated a) inner, that surface lying between two hemibranchs of the same holobranch; and b) outer, that surface lying between two separate holobranchs. The gill filaments were also equally divided into proximal, middle and distal portions.

For identification the monogenetic trematodes were stained in either Reynolds' Double Stain or Harris' Haematoxylin and mounted in Euperal (Turtox). Original descriptions were used for identifications and the current taxonomic status for each species is in accordance with Yamaguti, 1963.

The Chi-square test was applied to the data to determine if the parasites occurred on one region of the gill more than another. A Chi-square test was made between all arch subdivisions, regions of the arches, surfaces of the hemibranchs and divisions of the filaments unless specificity was obvious or small sample size made it impossible to test.

Results and Discussion

Seventy-six specimens of fishes were collected representing four species. Three of the four species, *Urophycis regius*, *Stenotomus chrysops* and *Orthopristis chrysopterus*, were parasitized by *Diclidophora maccallumi* (Price, 1943) Sproston, 1946, *Microcotyle stenotomi* Goto, 1900, and *Pseudotagia cupida* (Hargis, 1956) Yamaguti, 1963, respectively. *Peprilus triacanthus* was not infested. In Table 2 the total number of individuals, the number of inifested individuals, and the infestation rates are given for each species of fish. *U. regius* was most heavily parasitized.

Table 3 lists the morphological regions of the gills indicated in Figure 1, and gives the number of parasites of each species that was recovered from each region. Sample sizes for *M. stenotomi* and *P. cupida* were too small to apply a Chi-square test. *D. maccallumi* occurred in large enough numbers so that tests could be applied.

Chi-square tests indicated that site specificity existed at the 95% level of confidence at some of the regions (Table 4). The numbers of *D. maccallumi* occurring on arches I, II, and III are significantly higher than on arch IV. Since the difference in the numbers of parasites between gill arches I and III was large (Table 3), and the sample size was small, they were tested at the 90% level of confidence. The specificity at this level was narrowed to gill arches II and III. It is possible, that with a larger sample, a

Table 2. The percent of hosts infested and the mean number and range of parasites per host.

Host	Total No. of hosts	No. hosts infested	Infection rate % inf./tot.	Parasite	Total No. parasites	Mean No. para./host	Range of para./host
Urophycis regius (spotted hake)	42	20	47.6	*D. maccallumi*	166	8.3	1–14
Stenotomus chrysops (scup)	14	5	35.0	*M. stenotomi*	7	1.4	1–3
Orthopristis chrysopterus (pigfish)	10	1	10.0	*P. cupida*	1	1	—
Peprilus triacanthus (butterfish)	10	—	—	—	—	—	—

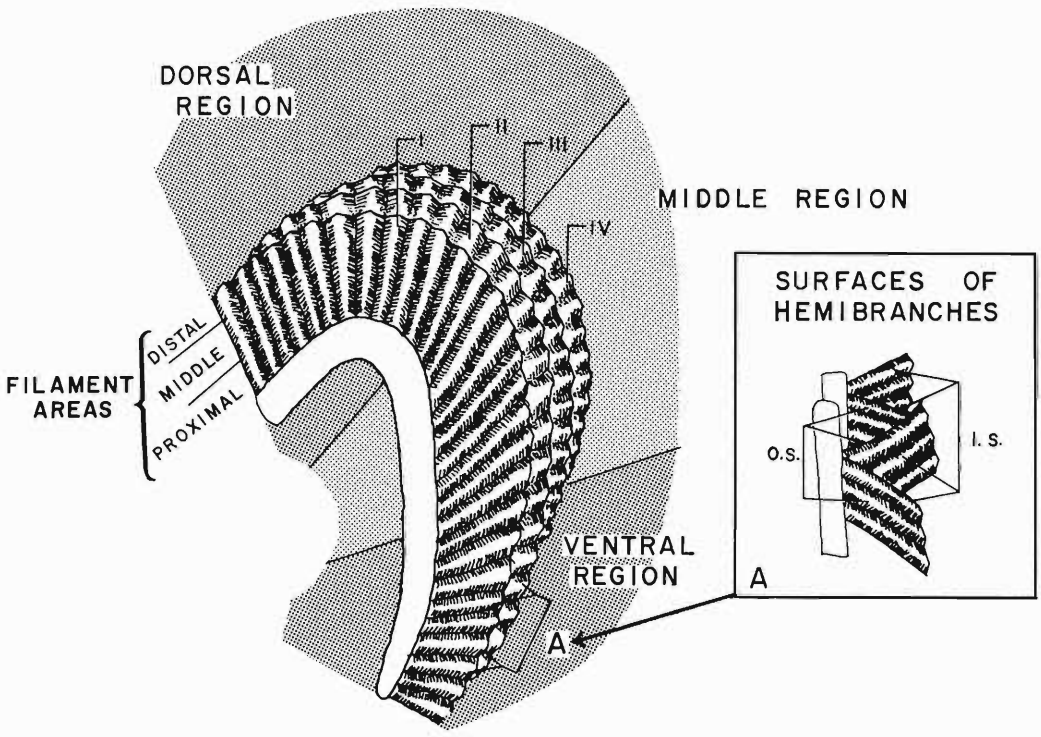

Figure 1. Illustration of the left side of the branchial basket showing the arbitrary divisions. O.S.—outer surfaces; I.S.—inner surfaces.

specificity for arches II and III would be indicated at the 95% level of confidence.

Site specificity is indicated for the middle and lower regions of the gill arches (Table 4). Since 80% of the attached specimens occurred on the inner surfaces of the hemibranchs specificity was obvious and no Chi-square tests were applied. Tests were applied to the inner surfaces of the lateral and medial hemibranchs but no significant differences were indicated (Table 4). Therefore, in all cases, the inner surfaces were "preferred" irrespective of the hemibranch.

Three times as many flukes were found on the middle region of the gill filament as on either the proximal or the distal regions. *D. maccallumi* was generally oriented with its posthaptor located proximally on the filament, and its haptoral clamps attached to lamellae on opposite sides of the same filament, apparently lying parallel to the filament in life (Figs. 2 & 3).

Llewellyn (1956) indicated that *Diclidophora merlangi*, from *Gadus merlangus*, occurred most often on gill arch I, and that *D. luscae*, from *G. luscae*, was more prevalent on gill arches II and III. These parasites were found with their posthaptors upstream to the ventilating current. Frankland (1955) indicated that *Dactylocotyle denticulata*, from *G. virens*, was more prevalent on the inner surfaces of the hemibranchs of gill arch I. Wiles (1968) found that *Diplozoon paradoxum* occurred most often on gill arches I and II in the bream (*Abramis brama* L.), on the inner hemibranch in the bream and minnow (*Phoxinus phoxinus* L.), and on the middle region of the gill arch in the minnow and roach (*Rutilus rutilus* L.). The adhesive attitude and site specificity of *Diclidophora maccallumi* are

Table 3. The distribution of parasites on the gills. Columns headed by a question mark (?) indicate numbers of parasites from undetermined locations.

Parasite	Total	No. of parasites on gill arches					No. of parasites on region of gill arch				Surface of hemibranchs Median		Surface of hemibranchs Lateral			Region of gill filament		
		I	II	III	IV	?	Dorsal	Middle	Ventral	?	In	Out	In	Out	?	Proximal	Middle	Distal	?
D. maccallumi	166	33	55	48	18	12	24	67	50	25	64	11	45	16	30	27	94	20	25
M. stenotomi	7	2	0	4	0	1	1	3	2	1	5	1	0	0	1	4	2	0	1
P. cupida	1	0	1	0	0	0	0	1	0	0	—	—	side		—	0	1	0	0

Table 4. The areas tested for *D. maccallumi* and the Chi-square values. Tests were run using the degree of freedom indicated in the parentheses at the 95% level of confidence.

Areas tested	Chi-square	Calculated Values
Gill arches I, II, III, IV	(3-df)	20.22*
Gill arches I and II	(1-df)	5.50*
Gill arches I and III	(1-df)	2.78
Gill arches I and IV	(1-df)	13.89*
Gill arches II and III	(1-df)	0.48
Gill arches II and IV	(1-df)	17.91*
Gill arches III and IV	(1-df)	13.66*
Regions of gill arch Dorsal, middle, and ventral	(2-df)	24.79*
Regions of gill arch Dorsal and middle	(1-df)	20.32*
Regions of gill arch Dorsal and lower	(1-df)	9.14*
Regions of gill arch Middle and lower	(1-df)	2.47
Hemibranchs Lateral and medial	(1-df)	3.82
Hemibranchs Inner lateral and inner medial	(1-df)	3.30

* Indicates significant difference.

similar to those described by Frankland (1955) for *Dactylocotyle denticulata*, Llewellyn (1956) for *Diclidophora merlangi*, and by Wiles (1968) for *Diplozoon paradoxum*, and may be influenced by the gill ventilating current as Llewellyn (1956) suggested.

An examination of the environmental factors influencing the microhabitat of *Diclidophora maccallumi* may yield a better understanding and possible explanation for the apparent site specificity. Hughes and Shelton (1957, 1958) working with *Salmo trutta* L., *Leuciscus rutilus* L., *Tinca tinca* L., and Saunders (1961) working with *Catostomus commersoni* (Lacépède), *Ictalurus nebulosus* (LeSueur), and *Cyprinus carpio* L. measured the hydrostatic pressure changes of the branchial pump during the respiratory cycle of these fishes. They found that during each cycle the flow of water from the buccal cavity to the opercular cavity was almost continuous and that for only a brief period during each cycle a back-pressure developed, reversing the direction of flow. Bijtel (1949), working with 12 species representing eight families of fishes, indicated that hemibranchs were spread during the respiratory cycle and the tips of hemibranchs on adjacent gill arches touched. He also described a coughing action which occurred periodically during the cycle. During this action muscles in the filament contracted pulling the hemibranchs together, the operculum was closed rapidly, and water was flushed backwards through the

Figure 2. A dorsal view of *D. maccallumi* attached to the inner surface of a hemibranch of *U. regius*. The posthaptor is attached proximally and the body lies parallel to the filament (preserved specimen).

Figure 3. A view of the haptoral clamps of *D. maccallumi* as they appear from the outer surface of the hemibranch of *U. regius* (preserved specimen).

gills. This action apparently served a cleaning function.

The direction of the ventilating current and the position of the hemibranchs during respiration may influence the position of *D. maccallumi* on the gills. The number of animals attached to the outer surface of the hemibranchs was small. This was possibly the result of the coughing action. This coughing action would tend to remove both young attaching forms and adults. If the invasion route of the parasite were passive, through the mouth, the brief backwash period in each respiratory cycle would offer an opportunity for new forms to attach to the exposed inner surface on the hemibranch. If the invasion route were active, through the operculum against the ventilating current, the spread inner surfaces of the gills would be the first surfaces encountered. In either case, the filaments of the hemibranchs appear to be capable of providing some protection from the almost continuous force of the ventilating current. The adduction of the hemibranchs during the coughing action might offer additional protection to those animals attached to the inner surfaces of the hemibranchs. Therefore, the inner surfaces of the hemibranchs would appear to be the more favorable site of attachment.

The fact that *D. maccallumi* occurs on the outer surfaces of the hemibranchs at all seems unusual. Llewellyn and Tully (1969) indicated that *D. macruri* is the only other *Diclidophora* studied that occurs on these outer surfaces. The unusual ability of *D. maccallumi* and *D. macruri* to attach to these surfaces may be accounted for by the structure of their posthaptoral clamps. A detailed study of these clamps and their method of attachment is necessary to gain a greater understanding of this phenomenon.

Diclidophora maccallumi occurred most often on gill arches I, II, and III. Paling (1968) working with *Salmo trutta* L., using glochidia of *Anodont acygenea* as indicators, determined

that the greatest volume of water in the gill ventilating current passed over the second and third gill arches. The first gill arch received the next greatest volume and the fourth the least. The distribution of *D. maccallumi* on the gill arches appears to vary directly with the distribution of the volume of the gill ventilating current. Apparently the greater volume of water flowing over the first three gill arches gives more parasites the opportunity to attach to these gill arches.

The indicated specificity for the middle and lower regions of gill arches could be attributed to the morphology of the branchial basket and the ventral position of the opercular opening. Larvae could come in contact with the lower region first during the brief backwash period and, therefore, would occur in high numbers in the middle and lower regions.

A higher number of *D. maccallumi* occurred on the middle region of the filament. This could have resulted from a need to maintain the mouth in a feeding position at the distal end of the filament as Frankland (1955) suggested for *Dactylocotyle denticulata*. The upstream position of the haptor and the resulting body position, seemingly parallel to the gill filament in life, may be, as Llewellyn (1956) suggested, an obvious adaptation that reduces resistance to the gill ventilating current.

Limited mobility of *Diclidophora* larvae (Frankland, 1955) and the knowledge resulting from this study would seem to indicate that any gill site specificity of *Diclidophora maccallumi* is primarily the result of the force and direction of the ventilating current and not selection on the part of the parasite. This is in accord with a similar suggestion made by Llewellyn (1956).

It must be remembered that only some of the physical factors which seem to influence site specificity have been discussed here. Physiological, behavioral, and further ecological studies are needed to provide a more complete picture of this type of host–parasite relationship.

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Comparative Development of *Ascaris suum* in Rabbits, Guinea Pigs, Mice, and Swine in 11 Days

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ABSTRACT: A comparative study was made of the development and migratory patterns of *Ascaris suum* in mice, guinea pigs, rabbits, and swine. Host animals were each given a single dose of 1,300 infective eggs and then killed 1, 2, 3, 4, 7, 9, or 11 days after infection (DAI). In mice, the infection essentially terminates 4 DAI with the attainment of middle third-stage in the liver, although few larvae migrate to the lungs where a few advance to late third stage. In guinea pigs, significant numbers develop to late third-stage but no farther in the lungs 7 DAI and very few migrate to the intestine. In rabbits, development was practically identical to that in swine in that early fourth-stage appeared in the intestine 11 DAI.

In previous papers on morphogenesis of *Ascaris suum* to the fourth stage in swine (Douvres, Tromba, and Malakatis, 1969) and in vitro (Douvres and Tromba, 1970) we discussed the comparative development of *A. suum* in normal versus abnormal situations. These studies and some observations on the development of *A. suum* in rabbits (Douvres and Tromba, 1966; Douvres et al., 1969) mice, and guinea pigs, led us to conclude that stage identification based on size, location in the host, or number of days of development was unreliable.

Our survey of some recent papers illustrates the confusion arising when the above criteria have been variously interpreted by different investigators. That is, larvae recovered from the lungs of guinea pigs were identified as follows: By depending on body lengths of less or more than 500 μ, larvae were, respectively, second and third stages, 4 or 5 days after infection (DAI) (Soulsby, 1961). By depending on location, larvae were third stage 8 DAI (Saz et al., 1968), and third and fourth stages 7 and 8 DAI (Matov and Terzijski, 1968). In mice, Sinha (1967) characterized as second stage all larvae measuring less than 305 μ, and as third stage, those measuring 315 to 1,960 μ. He found that larvae remained in second stage in the liver up to 3 DAI and were in second and third stages in both the liver and lungs from 4 to 12 DAI. Bindseil (1970) identified all larvae recovered from the lungs of mice up to 4 DAI as second stage; and those recovered from the