

THE LARVAL DEVELOPMENT OF TWO SPECIES OF GASTROCOTYLID TREMATODE PARASITES FROM THE GILLS OF *TRACHURUS TRACHURUS*

By J. LLEWELLYN

Department of Zoology and Comparative Physiology, University of Birmingham

(Text-figs. 1-9)

Plymouth scad (horse-mackerel) *Trachurus trachurus* (L.) are known to harbour three species of monogenean trematode gill parasites, of which two belong to the Gastrocotylidae and one to the Microcotylidae. The oncomiracidia (= newly hatched larvae) of these parasites have already been described (Llewellyn, 1957*a*), but nothing is known of the developmental stages intervening between the oncomiracidia and the adults. Bychowsky (1957) has stated that some information is available about the larval development of 13 monogenean families, leaving 15 families, including the Gastrocotylidae, about whose larval development nothing is known. The ontogenetic development of the Gastrocotylidae is of especial interest since the oncomiracidia are bilaterally symmetrical, but the adults invariably show an extreme degree of asymmetry.

In spite of a rigorous search, no larval monogeneans were found on mature *Trachurus* (20-30 cm long) at Plymouth during July and August 1954-8. Even in smaller scad (10-15 cm) examined in the same months in 1957 and 1958, all the parasites were found to be mature and egg-laying.

On 6 May 1959 about 50 specimens of *Trachurus trachurus*, of 9.3 to 12.6 cm length, were landed at Plymouth and sent in ice to Birmingham. Here 19 out of a sample of 20 of these young scad were found to bear an abundance of larval monogeneans, and immediately some new problems presented themselves: (*a*) is there a seasonal rhythm in the reproductive cycle of the monogenean parasites? and (*b*) do these particular monogenean larvae become parasitic only on young scad? It was of course realized that these questions would be best answered by continuous observations over a long period, but since this was impracticable, it was thought that some indications might emerge from comparing the parasite populations from hosts of different ages. Accordingly, through the most helpful co-operation of the staff of the Plymouth Laboratory, some large *Trachurus trachurus* caught off Plymouth on 20 May were measured individually to permit estimates to be made of their ages, then deep-frozen, and later the heads and gills were sent in vacuum flasks to Birmingham.

The gills of all the fishes were searched carefully with a stereomicroscope, and the dead parasites were rinsed in sea water and preserved in 4% formaldehyde. Some specimens were stained in haematoxylin or carmine and mounted in Canada Balsam, and some were sectioned at 6μ , but most were mounted freely without pressure of a coverglass, in 4% formaldehyde, to permit measurements to be made.

The parasites were identified as *Gastrocotyle trachuri* van Beneden & Hesse (present relatively abundantly) and *Pseudaxine trachuri* Parona & Perugia (present relatively rarely); no microcotylids were found. A combined total of 182 larval and adult *Gastrocotyle* was found to be distributed among 24 host fishes as indicated in Table 1.

TABLE 1

Length of host (cm)	Probable age of host* (yr)	No. of host specimens examined	No. infested	Total no. of parasites	Mean no. of parasites/host	Mean and range of no. of clamps/parasite
Under 12.9	Up to 1	10	9	116	11.6	12.5 (0-21)
13.0-18.9	1-2	3	3	37	12.3	20.4 (9-35)
19.0-23.9	2-3	6	5	25	4.2	21.0 (0-36)
Over 24.0	Over 3	5	1	4	0.8	15.8 (12-17)

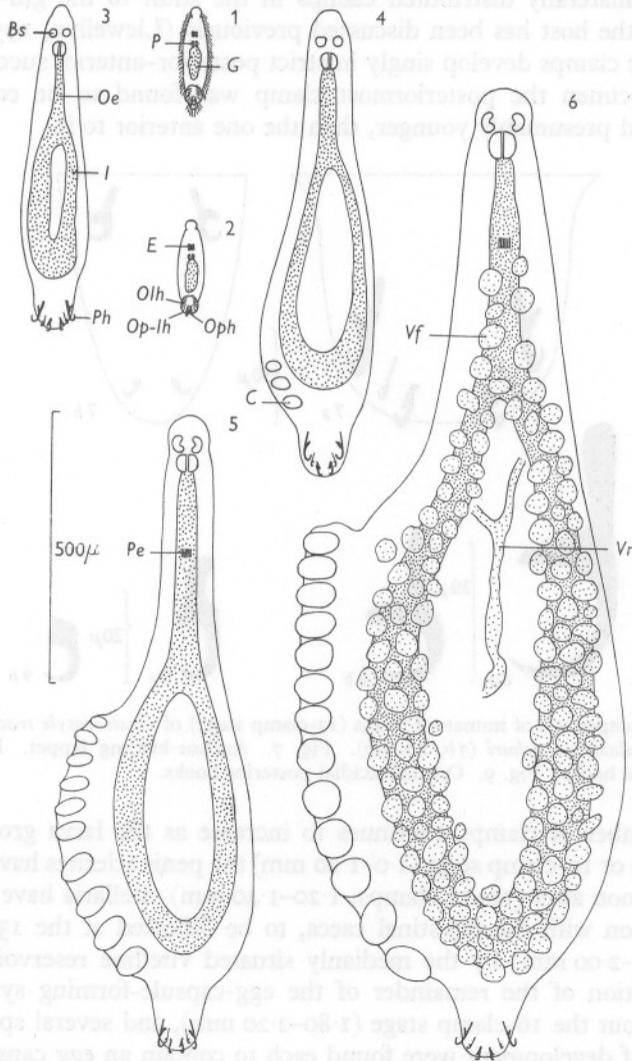
* Based on information kindly supplied by the late Dr G. A. Steven (Marine Biological Association of the United Kingdom, Report of the Council for 1957-58, p. 15).

THE LARVAL DEVELOPMENT OF *GASTROCOTYLE TRACHURI*

The oncomiracidium, about 0.16-0.20 mm in length (Figs. 1, 2) has been described previously (Llewellyn, 1957*a*), but subsequent observations during the course of abortive attempts to infect adult *Trachurus* with oncomiracidia have shown that the postero-lateral hooks may be as long as 26μ , and a more accurate expression of their size range is 23μ (19-26 μ). The posterior hooks are 26μ (23-28 μ) in length.

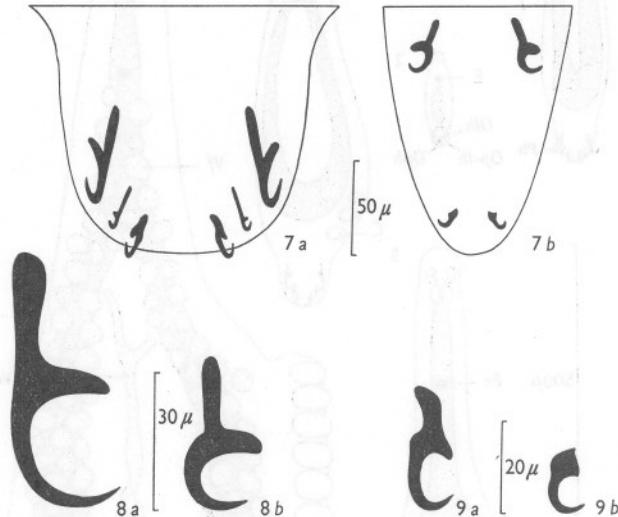
The earliest post-oncomiracidial larvae found were bilaterally symmetrical and about 0.50-0.85 mm long (Fig. 3). These larvae have lost the eyes and the 4 pairs of lateral hooks of the oncomiracidium, the place of the lateral hooks being taken, functionally if not topographically, by a pair of relatively large hooks each 54μ (52-56 μ) long (Figs. 3, 8*a*). These hooks in *Gastrocotyle* obviously correspond to those hooks in *Microcotyle labracis* which I described as 'primordial adult hooks' (Llewellyn, 1957*a*). It is noteworthy that all three pairs of hooks (i.e. the oncomiracidial postero-lateral and posterior hooks, and the newly acquired 'large hooks') present in *Gastrocotyle* at this stage persist without change of size or shape throughout all succeeding larval stages and survive in the definitive adult on the 'anchor-bearing lappet' (Fig. 7*a*) that has been described in many monogeneans. The alimentary canal, which in the oncomiracidium consisted merely of a mouth, pharynx, and simple sacculate intestine, is now provided with a pair of buccal suckers; the gut is differentiated into an oesophagus that bifurcates into two intestinal

limbs and these become confluent posteriorly. The walls of the oesophagus and the intestine are lined by scattered pigment cells indicating, by analogy with what is known about similar cells in adult polyopisthocotylineans, that the larvae have already been feeding on blood (Llewellyn, 1954).



Figs. 1-6. The larval development of *Gastrocotyle trachuri*; all diagrams drawn to the same scale. Fig. 1. Newly hatched oncomiracidium. Fig. 2. Oncomiracidium after shedding of ciliated epidermis. Fig. 3. Bilaterally symmetrical post-oncomiracidial larva. Figs. 4-6. Immature stages with 3, 10, and 13 clamps respectively. Bs, buccal sucker; C, clamp; E, eye; G, gut; I, intestine; Oe, oesophagus; Olh, Op-lh, Oph, lateral, postero-lateral, and posterior hooks respectively of oncomiracidium; P, pharynx; Pe, penis; Ph, post-oncomiracidial hook; Vf, vitelline follicle; Vr, vitelline reservoir.

During the next phase (0.60–1.00 mm) asymmetrical development begins: clamps develop on one side only of the posterior region of the body, which becomes slightly wider to accommodate them (Fig. 4). There is a complete absence of clamp development on the other side of the body. The relationship of these unilaterally distributed clamps in the adult to the gill-ventilating current of the host has been discussed previously (Llewellyn, 1956, 1957*b*). Usually the clamps develop singly in strict posterior–anterior succession, but in one specimen the posteriormost clamp was found to be considerably smaller, and presumably younger, than the one anterior to it.



Figs. 7–9. Comparison of immature forms (13-clamp stage) of *Gastrocotyle trachuri* (7*a*, 8*a*, 9*a*) and *Pseudaxine trachuri* (7*b*, 8*b*, 9*b*). Fig. 7. Anchor-bearing lappet. Fig. 8. Post-oncomiracidial hooks. Fig. 9. Oncomiracidial posterior hooks.

The number of clamps continues to increase as the larva grows bigger, and at the 9 or 10-clamp stage (1.0–1.20 mm) the penis sclerites have appeared (Fig. 5). Soon after (10–12 clamps, 1.20–1.40 mm) vitellaria have developed in association with the intestinal caeca, to be followed at the 13–16-clamp stage (1.50–2.00 mm) by the medianly situated vitelline reservoir (Fig. 6). The formation of the remainder of the egg-capsule-forming system takes place at about the 16-clamp stage (1.80–2.20 mm), and several specimens at this stage of development were found each to contain an egg capsule which, however, appeared to be without embryonic (ovum/oocyte/zygote) contents. In fact, no germarium could be identified in whole mount preparations, and so paraffin sections of 13-, 14-, 15- and 16-clamp larvae were prepared. The specimens, having been preserved in ice before histological treatment, yielded comparatively poor sections, but it was possible to determine that the

testes and germarium were still immature. The inference is, then, that the egg-capsule-forming apparatus (the vitellarium and the ootype and its associated glands) becomes functional before the truly germinal portions of the genitalia (the testes and germarium). Egg capsules yielding oncomiracidia have been collected from a specimen of *Gastrocotyle* with 19 clamps. A continued steady increase in the total length of the body is accompanied by a correspondingly steady increase in the number of clamps until eventually a maximum of 35 to 40 clamps is present in parasites of about 2.80–3.20 mm long.

THE LARVAL DEVELOPMENT OF *PSEUDAXINE TRACHURI*

The larval development of *Pseudaxine trachuri* is generally similar to that of *Gastrocotyle trachuri*, but the following differences have been found to occur.

Morphologically the oncomiracidium of *Pseudaxine* may be reliably distinguished from that of *Gastrocotyle* only by the smaller posterior hooks, which are 19μ ($18-20\mu$) in *Pseudaxine* and 26μ ($23-28\mu$) in *Gastrocotyle*. The difference in size of the postero-lateral hooks, which have been found to be 19μ ($18-20\mu$) in *Pseudaxine* and 21μ ($20-26\mu$) in *Gastrocotyle*, is likely to be a less reliable distinction, especially since the postero-lateral hooks of *Pseudaxine* are destined to reach a length of $24-26\mu$ before they eventually disappear.

With the acquisition of the post-oncomiracidial hooks the two species of larvae may be readily distinguished from each other: in *Pseudaxine* these hooks are 32μ ($31-33\mu$) long whereas those of *Gastrocotyle* are 54μ ($52-56\mu$) long. The shapes of these hooks are also different in the two parasites (Fig. 8).

While in *Gastrocotyle* the posterior hooks retain their oncomiracidial size and form without any alteration throughout development, in *Pseudaxine*, between the 5- and 12-clamp stages, the proximal regions of the posterior hooks disappear so that these hooks decrease in length from 19μ to about 12μ , and assume a different shape (Fig. 9*b*). The posterior hooks of *Pseudaxine* persist in this reduced form throughout adult life. While the reduction in size of the posterior hooks of *Pseudaxine* is taking place, i.e. between the 5- and 12-clamp stages, the postero-lateral hooks are lost completely.

Whereas the positions relative to each other of the oncomiracidial and post-oncomiracidial hooks remain constant throughout life in *Gastrocotyle*, in *Pseudaxine* the 'anchor-bearing lappet' elongates considerably so that in the adult the surviving oncomiracidial posterior hooks and the post-oncomiracidial hooks are relatively further apart (Fig. 7*b*).

Other features of the larval development of *Pseudaxine* are generally similar to those of *Gastrocotyle*.

DISCUSSION

Both larvae remain bilaterally symmetrical for a considerable period of post-oncomiracidial development, sufficient for the acquisition of buccal suckers and a new pair of large hooks. It is possible that this period could be used to investigate experimentally the factors, environmental (unilateral incidence of the gill-ventilating current, see Llewellyn, 1957*b*) or genetical, which determine the side of the subsequent asymmetrical development of the clamps.

In both species a pair of relatively large hooks develops in the first post-oncomiracidial stage. There is no trace of these in the newly hatched larvae. Bychowsky (1957) expressed the opinion that the corresponding hooks in the Microcotylidae and Mazocraeidae were not homologous with any of the six pairs of hooks present in the larvae of the majority of diclidophorideans, and, quite independently, I referred to such hooks in *Microcotyle labracis* not as larval hooks, but as 'primordia of adult hooks' (Llewellyn, 1957*a*). The present study supports the view that these 'large hooks' are post-oncomiracidial, but since they are relatively most prominent and have reached their definitive size during early larval development, namely, after the loss of the oncomiracidial lateral hooks, and before the development of the clamps of the adult, these hooks are probably best regarded as essentially larval features. Their appearance during the embryonic development of *M. labracis* is probably no more than an example of the kind of precocious development met elsewhere in polyopisthocotyleans, e.g. the appearance in the embryo of *Diplozoon paradoxum* of the first pair of clamps of the adult (Zeller, 1872).

The finding of larval and immature stages of *Gastrocotyle* and *Pseudaxine* in May, but of only adults in July and August, suggests that a seasonal rhythm is present in the reproductive cycle of these parasites.

The recovery from hosts of over two years of age of four immature specimens of *Pseudaxine* and 15 immature specimens of *Gastrocotyle*, one of which was a very early larva at the stage before any clamps had developed, indicates that it is not only 'young' (under two years old) *Trachurus* which are susceptible to infestation by these monogeneans, but older fishes also.

The main difference between the larval development of *Gastrocotyle* and *Pseudaxine* and those of *Microcotyle spinicirrus* as described by Remley (1942) and *Diclidophora denticulata* as described by Frankland (1955) is the survival of the 'hook-bearing lappet' with at least some of its hooks in the first two species, and its complete disappearance in the other two species.

I am happy to acknowledge the help I have received from the Director and Staff of the Plymouth Laboratory, and especially that given by Mr J. E. Green and Mr A. D. Mattacola.

SUMMARY

Larval forms of *Gastrocotyle trachuri* and *Pseudaxine trachuri* are common on *Trachurus trachurus*, especially on young fishes, at Plymouth in May, but only adult parasites are found in July and August.

The larval development of these monogeneans includes a bilaterally symmetrical post-oncomiracidial stage in which the most prominent adhesive organs are a pair of relatively large post-oncomiracidial hooks. Asymmetrical development begins with the formation of the principal adult adhesive organs, the clamps.

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