

THE BIOLOGY OF A COMMENSAL COPEPOD,
ASCIDICOLA ROSEA THORELL, IN THE
ASCIDIAN *CORELLA PARALLELO-*
GRAMMA (MÜLLER)

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(Text-figs. 1 and 2)

The considerable advantages offered by ascidians as hosts to small commensal crustaceans are clearly implied by the large number of ascidicolous copepods which have been described; and the attractions of this particular niche are further evidenced by the fact that these copepod mess-mates are of polyphyletic origin and cosmopolitan distribution.

The study of such associations, however, has been limited almost entirely to a bare note of their existence, although the papers of Canu (1892) and Chatton & Brément (1915) are partial exceptions.

That ascidians so frequently act as hosts is not in itself surprising. Their comparative immunity from predation, their maintenance of a feeding current and the capaciousness of many of their internal cavities afford protection, accessible food and a certain freedom of movement. Furthermore, the elaborate ascidian mechanism of food intake and concentration offers, in its several stages, different possibilities of utilization to copepod commensals of varying structure and mobility. Thus, in the pharynx alone, a commensal might exploit the particles entering the oral aperture; or it might seek these as, trapped in the moving sheets of mucus, they are slowly swept towards the dorsal lamina; or, yet again, the concentrated food string, *en route* to the oesophagus and stomach, might be the focus of attention. Encompassed, therefore, by this complex nutritional mechanism many variously adapted copepods find optimal living conditions.

In any attempt to shed some light on associations of this type, three conditions must be satisfied. The chosen copepod species must be of relatively large size, the ascidian concerned must possess an unusually transparent test, and observations must be made in a fairly large volume of undisturbed sea water to ensure normal behaviour on the part of the host. After a prolonged search for suitable material in Strangford Lough, Co. Down, it was found that the association between *Ascidicola rosea* Thorell and *Corella parallelogramma* (Müller) proved almost ideal. The female *Ascidicola rosea* attains a length of over 4 mm, while the glass-clear tunic of *Corella parallelogramma* is perhaps

its most striking feature. A Perspex tank, coupled with a viewing system of lenses and ground-glass screens, with adequate background lighting, solved most of the problems of observation under nearly normal conditions.

GENERAL CONSIDERATIONS

C. parallelogramma, a solitary ascidian belonging to suborder Phlebobranchiata, occurs sparingly around the British coasts, being found attached to stones, weed, shells, etc., usually in clear offshore waters down to depths of 150 m (Berrill, 1950). Abroad, its range includes a large part of the Scandinavian coast and the Mediterranean. It attains a length of some 5 cm, large specimens being easily dredged in about 12 m in Strangford Lough, where the species is abundant on suitable ground, especially during the autumn months. As well as *Ascidicola rosea*, *Corella* is known to harbour several other ascidicolous copepods—*Notodelphys agilis* Thorell, *N. caerulea* Thorell, *Doropygus porcicauda* Brady, *D. pulex* Thorell, *Pachypygus gibber* Thorell, and the cyclopoids *Ascomyzon lilljeborgi* Thorell and *Lichomolgus albens* Thorell, the last-named sometimes cohabiting with *Ascidicola*. At the beginning of October, *A. rosea* was present in about 25% of *Corella* examined; three weeks later 60% of the hosts were infected.

Ascidicola rosea is usually classified as the sole member of the family Ascidi- colidae, suborder Notodelphyoida. In a recent paper, however, Lang (1948) has suggested that this very heterogeneous suborder should be split up, in which case the Ascidi- colidae, along with several other notodelphyoid families, should be regarded as a special tribe of gnathostomatous cyclopoids—a taxonomic realignment which has much to recommend it. This copepod has been reported from several localities around Britain and Ireland, while abroad it is found on the Norwegian coast as well as in the Baltic and Mediterranean. In addition to *Corella* its hosts include *Halocynthia papillosa* (L.), *Ascidia mentula* (Müller), *A. obliqua* Alder, *Ascidiella aspersa* (Müller), *A. opalina* M'Gill, *Ciona intestinalis* (L) and *Phallusia* sp. I have also obtained a single female specimen from *Pyura squamulosa* (Alder).

During the winter months, the *Corella* population of Strangford undergoes a very marked diminution. Since the commensal breeds throughout the year, the copepodids must therefore seek alternative hosts during the colder months in order to complete their development. *Ascidiella aspersa*, which remains plentiful during the critical period, is a frequent choice, and it seems likely that it is this species which carries the main bulk of the overwintering *Ascidi- cola* population.

Until recently, the genus *Ascidicola* was thought to be monotypic and restricted to the northern hemisphere, but Lang (1949) has described a second, closely allied species, *A. aculeoretusa*, from *Pyura georgiana* Mchlsn. taken in 250 m off South Georgia.

POSITION AND FEEDING ACTIVITIES OF THE COPEPOD

Schellenberg (1921) summarized in tabular form the then available information regarding the site occupied by *Ascidicola rosea* within its various ascidian hosts, and the occasional records of the past thirty years have added to his list little of material value. In most cases, it is reported to occur in the pharynx, although a minority of observers have collected specimens from the stomach and intestine. Examination of infected living *Corella*, however, leaves little doubt that (in this host at least) *Ascidicola's* normal 'operational base' is neither pharynx nor stomach, still less the intestine, but the oesophagus. The stomach is entered only in certain circumstances, while excursions into the pharynx proper are rare indeed. On only one occasion, in fact, was *Ascidicola* observed to cross the pharyngeal wall.

The preference shown for the oesophageal region is explained by the proximity of the food string descending the dorsal lamina, for the particles entrapped on it appear to provide this copepod with its only source of nourishment. During active feeding, *Ascidicola* grasps the mucus cord with the four anterior pairs of pereopods and climbs up it for a short distance, feeding intermittently. Although as much as two-thirds (or, exceptionally, the whole) of the body may project head first into the pharynx, the slow downward movement of the food string as it passes into the ascidian's stomach tends continually to draw the copepod back into the oesophagus. After sinking a short distance in this way, *Ascidicola* adjusts its position by another climbing movement. The initiation of a fresh ascent often appears to follow contact between the urosome of the passively descending commensal and the curving wall of the oesophagus where the latter narrows before entering the stomach. During its ascent, *Ascidicola* may climb in a straight path or adopt an irregular side to side course. Not infrequently a complete spiral is achieved, although this is probably due in part to the twisting of the cord itself. While on the food string, the copepod twitches its antennules intermittently and various unidentifiable movements of the antennae and mouthparts can be seen. Occasionally it may tug at the food string with a vigorous movement of the head, sometimes producing a noticeable kink in the mucus cord. Whether this action is designed to remove an attractive food particle or to accelerate the downward passage of the still unexplored upper portion of the string, it is difficult to say. Evidence in support of the latter interpretation may be provided by the fact that this movement generally takes place when the food string is thin and poorly developed. Feeding activities may cease for short periods, the copepod withdrawing its head and anterior segments from contact with the cord and remaining motionless in an attitude somewhat resembling that of a resting sphingid moth larva. Climbing activity appears to be greatly reduced in the presence of a very thick food string, the commensal remaining almost motionless and pressed against the oesophageal wall.

When active feeding is not taking place, *Ascidicola* lies cradled in the oesophageal bend, usually with the ventral surface uppermost. Since, in this position, the food string is passing in close proximity to the mouthparts, it is possible that some feeding still occurs. Close observation, however, indicates that this must be on a reduced scale, if in fact it takes place at all.

Feeding activity appears to be of a non-cyclical nature. Certainly no daily periodicity is involved, since copepods may still be seen on the food string several hours after darkness has fallen as well as during the day. The periods of quiescence, therefore, spent cradled in the oesophageal curve, in most cases probably follow optimal feeding on a rich food string, thus establishing an intimate link with the trophic activity of the host.

Under laboratory conditions, *A. rosea* will often adopt a third position, lying in the oesophagus with its head towards the ascidian's stomach. When in this attitude, the copepod may bend the terminal segment of the urosome upwards to an angle of 45° , in this way perhaps achieving a braking effect against the ciliary current of the oesophagus. This inverted position is very frequently associated with a poorly developed or completely interrupted food string, and suggests that the commensal turns to follow the final truly nutritive section of the cord as it passes into the digestive regions of the host's canal. Often this attitude is maintained for long periods, *Ascidicola* sometimes seeming to grasp and halt the receding portion of rich food string before it slowly vanishes into the host's stomach. In this way the stomach may be actually entered for varying periods of time, although in the natural state it is probably seldom necessary to seek food in this region. An interruption of the host's normal feeding pattern, consequent upon its capture, may well account for some of the reported instances when *Ascidicola* has been found in an ascidian's stomach. (There is, however, some slight evidence that rather more time may be spent in this region when the host concerned is *A. aspersa*.) Only twice during the present study have copepods been seen in the intestine and twice in the rectum.

Two occasions on which the commensals were observed to enter the stomach are worthy of record. Once, after several ascents, the copepod remained quiescent on the descending food string and was eventually drawn, tail first, into the stomach. Some minutes later, after several exploratory movements, it emerged head foremost and once again mounted the food string. In another instance, following the introduction of a second *Ascidicola* which became trapped on the descending cord, the rightful occupant turned completely round and crawled into the stomach, followed passively by the immobilized specimen. A similar turning reaction sometimes takes place if an abrupt stimulus is applied—for example, focusing a concentrated spot of light on the copepod.

Several hitherto puzzling features of *A. rosea*'s anatomy become explicable in the light of its intimate association with the food string. Thus Sars (1921)

described the penultimate abdominal segment as 'having the ventral part of the hind edge remarkably thickened and densely clothed with small pricks'—a feature which was also noticed by Brady (1878). These 'small pricks' are in fact tiny sharply pointed spines, and since the region in which they occur is pressed closely against the mucus cord there can be little doubt that they aid in maintaining a firm grip, functioning, in fact, rather like a crampon (Fig. 1).

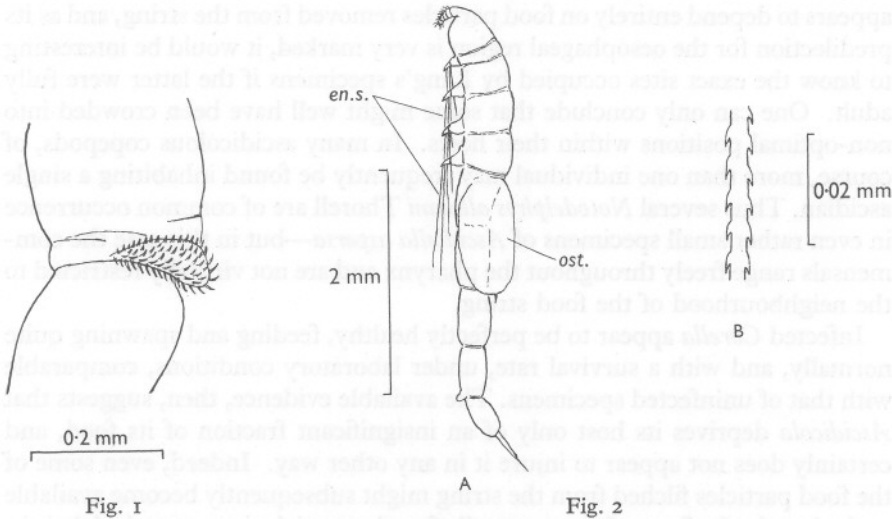


Fig. 1. Distal part of penultimate segment of female *Ascidicola rosea* to show the spinous pad. The proximal part of the anal segment is also shown.

Fig. 2. A, female *Ascidicola rosea* in lateral view to show the long endopodal setae (*en.s.*) and the left oostegite (*ost.*). B, a small portion of an endopodal seta, showing the spines with which it is beset.

During a climb, it is occasionally noticeable that the anterior urosome segments will extend telescopically, carrying the body upward, while the posterior two remain temporarily motionless in close contact with the food string, thus providing a point of leverage. The above-mentioned bending of the terminal urosome segment is also apparent during some climbs, although its object in this case is clearly to bring the spinous pad on the penultimate segment into more effective play.

The extraordinarily long setae carried by the endopodites of the four anterior pereopods are also of interest in a similar connexion. In life, their tips are directed posteriorly (Fig. 2A) and the entire length of each seta is applied to the main axis of the food string. In spite of Sars's assertion (1921) that these setae are quite smooth, examination under high power reveals them as beset with minute spines (Fig. 2B). It is possible that these spines, by engaging in the food string, likewise represent an adaptation to assist in maintaining a secure grip while in a vertical position on the cord.

RELATIONS WITH THE HOST

Although Lang (1948) records single specimens of *Corella parallelogramma* as harbouring up to five females of *Ascidicola rosea*, the rule in dredged ascidians from Strangford Lough seems to be one copepod per host. Occasionally, however, a very young female will be found with an adult, the former occupying a more anterior site on the food string. As this rather large commensal appears to depend entirely on food particles removed from the string, and as its predilection for the oesophageal region is very marked, it would be interesting to know the exact sites occupied by Lang's specimens if the latter were fully adult. One can only conclude that some might well have been crowded into non-optimal positions within their hosts. In many ascidicolous copepods, of course, more than one individual may frequently be found inhabiting a single ascidian. Thus several *Notodelphys allmani* Thorell are of common occurrence in even rather small specimens of *Ascidiella aspersa*—but in this case the commensals range freely throughout the pharynx and are not virtually restricted to the neighbourhood of the food string.

Infected *Corella* appear to be perfectly healthy, feeding and spawning quite normally, and with a survival rate, under laboratory conditions, comparable with that of uninfected specimens. The available evidence, then, suggests that *Ascidicola* deprives its host only of an insignificant fraction of its food, and certainly does not appear to injure it in any other way. Indeed, even some of the food particles filched from the string might subsequently become available to the host in the form of the copepod's faecal material, since organic debris is, of course, utilized by ascidians as well as living micro-organisms. While we are therefore faced with the usual, if artificial, difficulty of deciding on the precise ecological status of *Ascidicola*, its description as an endocommensal would seem to fit the known facts accurately enough.

REPRODUCTION

A. rosea carries its pink-coloured eggs in two thin oval masses protected by the dorsally situated lamellate oostegites (see Fig. 2A). These structures were regarded by Canu (1892) as the enlarged and modified fifth pair of pereopods, although his interpretation was later disputed by Chatton & Brément (1915), who maintained that they could equally be akin to the alate processes of the notoapterophorids. Their exact nature, therefore, may still be regarded as *sub judice*. Each egg-mass is slightly concave ventrally, being moulded to the contours of the body, and each contains approximately the same number of eggs. The actual egg number, however, may vary within rather wide limits. This variability may be governed, as Marshall & Orr (1952) have shown for *Calanus finmarchicus* (Gunnerus), by the food supply available to the parent, or alternatively may be correlated with the decreasing fertility of a female which has already, perhaps, produced several broods. Whatever the reason, an

egg-mass may contain as few as 20 or as many as 70 eggs. Individual eggs measure 0.13–0.14 mm in diameter.

The male, which attains little more than 1 mm in length, lacks the obviously specialized features of the mature female and is adapted for an active free-swimming existence. As one might expect, it is encountered much less frequently. Sars (1921) mentions having found only a single specimen, dredged in the free condition. None have been seen in ascidians from the Strangford area and it can probably be assumed that its occurrence as an adult in the host is in the nature of a fleeting visit only, during which mating takes place. The possibility, however, cannot be excluded that fertilization is performed by a male which had developed within the same host as a young female and which has subsequently left the ascidian, since Marshall & Orr (1955) have shown that in *Calanus* fertilization takes place at a relatively early stage of ovarian development. On the other hand, the fact that not one adult male has been detected within *Corella* during the Strangford investigation renders this a less likely explanation.

Perhaps the most interesting problem which arose during the study of this association concerned the mode of exit from the host of the copepod's young stages. Short of rupturing both mantle and tunic—a task manifestly beyond the powers of either eggs or nauplii—and in the absence of evidence that the peribranchial slits were utilized, the only available escape routes would be via either the oral or the atrial siphons. If the eggs hatched *in situ* on the parent copepod and the nauplii attempted to reach the outside world via the oral route, they would encounter not only the force of the inhalant current but the dangers attendant upon a journey through the mucus-lined pharynx—a structure specifically evolved for the capture of micro-organisms. To traverse the entire length of the food-laden alimentary canal, however, would involve an equally hazardous passage for these fragile forms. It seemed probable, therefore, that egress took place in the egg stage. This would, however, need very accurate timing, since to shed relatively large demersal eggs from the protection of the host long before hatching would unnecessarily present hostages to fortune in view of the ubiquity of benthic scavengers.

In the account which follows, although the initial part of the process has not been observed in detail, due largely to the opacity of the ascidian's stomach wall, it can be inferred beyond any reasonable doubt to take place as described. Some time before the eggs are due to hatch, the female leaves her position in the oesophagus and enters the stomach. Here the ripe egg-masses are detached from beneath the oostegites. (This occurs quite regularly in females extracted from the host.) Shortly after being deposited in the stomach, the inner membrane of each egg, with its contained nauplius, becomes free, leaving the cluster of adherent outer membranes to remain as a honey-combed ghost-like replica of the original egg-mass—again a noticeable occurrence with ripe isolated ovisacs. From the host's stomach, the nauplii—still sheathed in their

protective inner membranes—are carried in small or large groups through the intestine and up the rectum, in which region they can be clearly seen provided only a small quantity of food material is present in the host's gut. In one instance a cluster of some 30 eggs took 30 min to traverse the last 8 mm of the rectum. As the anus is reached, and the full strength of the exhalant current encountered, final rupture of the delicate enveloping membrane takes place and the freed nauplii are expelled with almost explosive force through the atrial siphon. In a few of the hatchings observed, a group of eggs became lodged in a small terminal 'pocket' of the host's rectum, where they remained for some hours, but whether this would occur in ascidians with a full gut is difficult to determine. Meanwhile the female copepod has re-entered the oesophagus, and a new batch of eggs has, in most cases, been squeezed into the space beneath her oostegites. Eighteen days elapsed between successive hatchings of nauplii from one female observed in the laboratory.

This method of egg dispersal via the food canal of the host has not, to my knowledge, been described in any other copepod—though it is quite possibly the usual method for many of the species living in simple ascidians. It is, in its way, quite as remarkable as the egg-depositing migration of another ascidicolous form, *Enterocolides ecaudatus* Chatton & Brément, in which the mature female burrows up from the depths of its compound ascidian host to leave its egg-masses at the surface of the colony (Chatton & Harant, 1924). It is interesting also to observe how the presence of the inner egg membrane (a common feature in many free-living copepods, as shown by Marshall & Orr, 1954) is here of vital adaptive significance, serving as it does to protect the fragile nauplius during its passage through the host's gut.

Although only a subsidiary part of the present study, the various larval stages of *Ascidicola* perhaps merit the following brief notes, though it must be emphasized that the times given are approximate only. There are four clearly defined naupliar stages. The first-stage nauplius measures between 0.191 and 0.197 mm and swims with the usual jerky movement, exhibiting a marked preference for certain light intensities. After about 21 h, at a laboratory water temperature varying between 10.9° and 16.9° C, it moults to the second nauplius, which measures 0.206 mm. Again after approximately 21 h, the third nauplius appears, measuring 0.211 mm. 24 h later, the final nauplius stage is found, which attains 0.224–0.229 mm in length. This instar persists for about 31 h before the moult takes place to the first copepodid, which measures 0.349 mm and possesses six segments in all. At this stage the three anterior pairs of swimming legs are developed, though the third pair are still somewhat rudimentary. After a further 54 h, the second copepodid appears. This shows an increase in length to 0.369 mm, and possesses seven segments as well as a fuller development of the third pair of legs. After swimming freely for some time it displays a different type of behaviour, seeking the bottom of the dish and remaining quiescent for lengthy periods. If a *Corella* is now

placed in the same vessel, on subsequent examination it will be seen to contain copepodids scrambling about on the food string in the oesophageal region. Although fuller confirmation is desirable that the second copepodid represents the infective phase, there is little doubt that the developmental pattern will prove similar to that of *Enterocola fulgens* van Beneden (see Canu, 1892), in which further moults to the adult female stage occur within the host after entry by the second copepodid. It seems highly probable that a period spent in the ascidian is obligatory for all copepodids, male as well as female, if one can judge by the universal demise of copepodids not provided with a potential host. It must be admitted that these stages have not been seen often in *Corella*, but as they are so small they could very easily be overlooked, even when specifically sought.

It is hoped at some later date to investigate the nature of the attraction which the host ascidian apparently exerts over the second-stage copepodid. It seems unlikely that intake by chance alone should exercise a controlling influence on a life cycle so perfectly attuned in all other respects to the survival requirements of this specialized commensal.

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SUMMARY

A study has been made of the feeding activities and reproductive behaviour of the copepod *Ascidicola rosea* within one of its ascidian hosts, *Corella parallelogramma*.

Ascidicola rosea feeds on particles which it removes from the food string as the latter is passing through the oesophagus of the host. When active feeding is not taking place, the copepod remains quiescent in the oesophageal bend. An inverted position within the oesophagus is sometimes adopted; this is generally associated with a meagre development of the food string. Certain peculiar structural features—notably the spinous pad on the penultimate segment and the long endopodal setae—are considered to be adaptations which assist the copepod while clinging to the food string.

The eggs of *A. rosea* are deposited in the ascidian's stomach and the nauplii pass through the host's alimentary canal still enveloped by the inner egg membrane. On reaching the anus, hatching takes place and the nauplii are expelled from the atrial siphon. There are four naupliar and two copepodid stages. Free-swimming life lasts for about 6 days.

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