# The functional significance of blood plexuses in the ecology of *Ophelia bicornis* Savigny

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## ABSTRACT

The anatomy of the anterior blood vascular system of *Ophelia bicornis* is described with especial reference to its plexuses of inflatable blind capillaries. The functional significance of these plexuses under the influence of systolic blood pressure and their roles in the provision of an efficient burrowing mechanism through relatively dilatent sand whilst, at the same time, helping to maintain an efficient transport system for dissolved substances is described and discussed within the context of the ecology of the polychaete. The study has been carried out by direct observation of the living worm, by dissection and by analysis of absorbed radioactively-labelled glutamic acid.

## RÉSUMÉ

**Signification fonctionnelle des plexus sanguins pour l'écologie d'*Ophelia bicornis* Savigny**

L'anatomie du système vasculaire sanguin antérieur d'*Ophelia bicornis* est décrite en considérant plus spécialement ses plexus de capillaires aveugles et dilatables. La signification fonctionnelle de ces plexus, sous l'influence de la pression sanguine lors de la systole, leur rôle dans la mise en oeuvre d'un mécanisme efficace pour l'enfouissement dans des sables relativement compacts et le rôle qu'ils jouent, dans le même temps, comme système de transfert des substances dissoutes, sont décrits et discutés dans le contexte de l'écologie du Polychète. L'étude a été conduite par observation directe d'animaux vivants, par dissection et par l'analyse d'acide glutamique radioactif absorbé.

## INTRODUCTION

The mechanisms of digging into, and travelling through, marine sediments by soft-bodied invertebrates has been studied at some length. Both in the bivalved molluscs (ANSELL & TRUEMAN, 1967; TRUEMAN, 1968) and in the annelids (CHAPMAN, 1950, 1958), it has been shown that effective locomotion is usually achieved by an interaction of the body-wall musculature and an internally-developed hydrostatic tension in a body fluid, usually that which fills the coelom.

The most extensive studies of burrowing in the Polychaeta have involved *Arenicola marina*. CHAPMAN and

NEWELL (1947) and TRUEMAN(1966a,b) have discussed the nature and mode of action of the hydrostatic system, whilst WELLS (1944, 1952, 1954, 1961) has described the mechanical activities of the proboscis and their significance to the behaviour of the worm. The consensus of this work is that the primary thrusting force is generated by the interaction of the internal coelomic fluid pressure and the highly-controlled body- wall muscles and muscular, eversible proboscis.

WELLS (1954) and CHAPMAN (1958) pointed out that much of the success of this process is due to the presence in *Arenicola* of an open coelom which results in the deployment, at the anterior, of the total force generated by extensive areas of body-wall musculature reacting against a "whole-body" hydrostatic skeleton. In association with this, WELLS (1954) also suggested that the three retained anterior septa of *Arenicola* serve to resist the at-rest coelomic pressure in order to restrict unnecessary extension of the proboscis.*Ophelia bicornis*, too, has a coelom which is continuous throughout the body and two retained anterior septa, but they are used in a manner which is totally unlike that of *Arenicola*. This paper reports the mode of action of that coelomic apparatus and of the way in which it is augmented by the activities of the complex blood vascular system.

## MATERIALS AND METHODS

a. Anatomical and behavioural study.

*Ophelia bicornis* was collected from Bull Hill Bank in the estuary of the River Exe, Devon, U.K. Worms were killed and fixed by immersion for about three hours in 5% neutral formalin in sea water, some after fresh-water narcotisation, others without pre-treatment. This was necessary since, as reported by BROWN (1938) for *Ophelia rathkei*, the only agent which is effective for the narcotisation of *O. bicornis* is fresh water. Immersion in fresh water for about twenty minutes induces total narcosis and also causes the worm to extend to its full, straight, length. Since this is clearly induced by osmotic influx of water into the coelomic compartment, all dissections were performed on narcotised specimens for clarity and repeated on untreated specimens for confirmation of the exact state of the organs and tissues. In practice, it was found that even the most delicate coelomic capillaries were not modified by the fresh-water treatment.

Locomotory activities were observed in *Ophelia* provided with semi-transparent artificial sand composed of a mixture of glass beads of appropriate granularity moistened with sea water. Use of immature worms, with relatively unpigmented and thin body-walls, permitted a certain amount of direct observation of internal activity.

b. Amino acid uptake.

Amino acid uptake was investigated using L-[U-$^{14}$C] glutamic acid (50μCi. cm$^{-3}$), supplied by Amersham International, in a laboratory régime based upon "Analar" quality acid-cleaned sand and artificial sea water as formulated by DAWSON et al (1969).

132 μl of radioactive amino acid were added to 75ml of artificial sea water. This was thoroughly mixed and added to 150 g of sand and cooled to 10 °C. Experiments were carried out at this temperature. Sand prepared in this way was found to contain sufficient radioactive material to give 39 072 dpm. g$^{-1}$ sand at the degree of dampness used in the experiments (dpm = disintegrations. min$^{-1}$ as counted in the liquid scintillation counter).

*Ophelia bicornis* were placed in the prepared sand and allowed to burrow naturally.

Individual worms were removed at intervals of 10 min, 20 min, 30min, 45 min and 60 min, rinsed in artificial sea water and immediately cut into three portions representing the prostomial and pharyngeal region, the mid-gut (intestinal) region and the hind-gut (chiefly rectal) region. The mass of these portions was determined and they were prepared for liquid scintillation as follows.

The tissue was placed in glass scintillation vials with 0.9 ml of "Soluene 350" and digested at 40 °C overnight. After cooling to room temperature, 0.2 ml propan-1-ol and 0.4 ml 30 % hydrogen peroxide in two 0.2 ml portions over two hours, were added to decolorize the pigmented solution. After the addition of the second portion of hydrogen peroxide, the samples were heated to 40 °C to drive off excess peroxide and again allowed to cool.

5.0 ml "Packard 299" scintillation fluid were added to each vial and the activity of the mixture was measured in a Packard "Tri-carb 460 CD" liquid scintillation counter by the sample channels method against a quench curve prepared from measurement of vials containing "Packard 299" fluid with chloroform as the quench reagent.

c. Autoradiography.

Worms destined for autoradiographic study were allowed to ingest the radioactive amino acid-treated sand for two hours prior to fixation. This relatively lengthy treatment time was decided upon since the aim of the present

investigation was solely to determine the extent of amino acid uptake and its broad distribution within the tissues of *Ophelia bicornis*.

Worms were fixed in 3 % neutral formalin in sea water without prior narcotisation and, during fixation, were divided into 1.0 cm long portions so that the intestine could be cleared of sand. The fixed tissues were dehydrated through a standard ethanol series, embedded in paraffin wax and sectioned at 10μm. Sections were mounted on gelatin-subbed slides as recommended by Kodak Ltd (1978) and ROGERS (1979). They were coated with "Kodak" AR10 stripping film and allowed to expose, in the dark, at 15 °C for 14 days. The slides were developed in "Kodak" D-19 and fixed in "Kodak" F-5.

The *Ophelia* tissues acquired a pale straw colour during processing which rendered them clearly visible beyond the developed silver, thus obviating the need for staining.

Control of the technique was effected in two ways: a) by comparison with similarly- processed sections of tissues which had not been subjected to radioactive labelling and, b) by examination of putative developed silver grains by dark-field microscopy, as recommended by ROGERS (1979).

## RESULTS

a. The anterior coelom and its vascular system

The overall pattern of the blood vascular system followed the descriptions of CLAPARÈDE (1870) from *Ophelia radiata* and of BROWN (1938) from *O. rathkei*. The constructional design is in two major parts, a posterior system based upon a series of sinuses and an anterior system of complex blood vessels.

The whole length of the intestine of *O. bicornis* is surrounded by voluminous dorsal and ventral blood sinuses, of which the dorsal is the larger. The ventral sinus more nearly resembles an inflated blood vessel and, for most of its length, is virtually buried within the ventral tissues of the typhlosole.
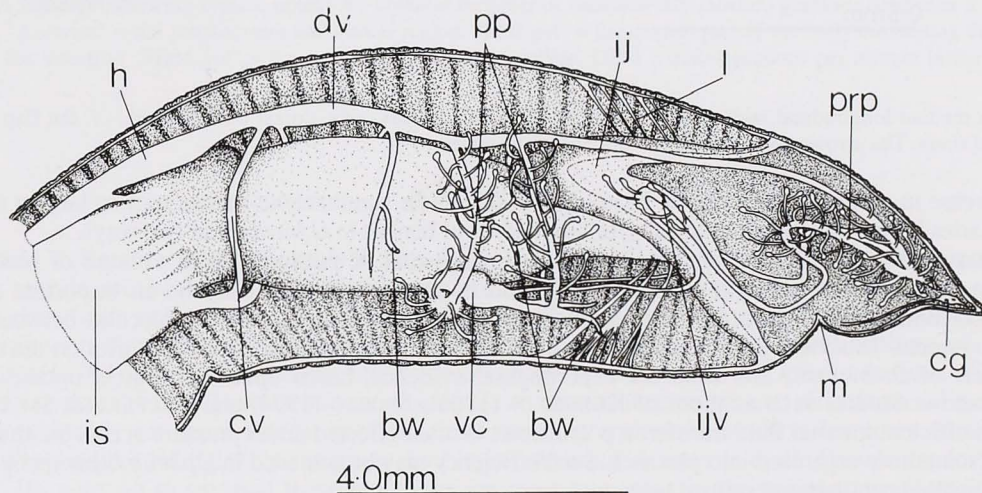


FIG. 1. — A semi-diagrammatic drawing of the anterior of *Ophelia bicornis* dissected from the right side and cut back to beyond the ventral nerve cord. For the sake of clarity the prostomial part of the pharynx is not shown. bw, vessels to the body-wall. - cg, cephalic ganglion. - cv, commissural vessel. - dv, dorsal blood vessel, - h, heart. - ij, "injector organ". - ijv, "injector organ" blood vessel. - is, intestinal sinus. - l, ligaments. - m, position of mouth. - pp, pharyngeal capillary plexus. - prp, prostomial capillary plexus. - vc, ventral cisterna.

The anterior blood system is illustrated semi-diagrammatically in Fig.1. The conspicuous parts of the system arise from the junction of the buccal chamber with the intestine (shown at the left of Fig.1). At this point, the dorsal intestinal sinus gives rise to a muscular heart which contains a non-return flap-valve (Fig.2). From the heart, an anterior dorsal vessel gives rise to four paired vessels. From posterior to anterior these are - a pair of lateral vessels which connect the dorsal and ventral sinuses, a pair of vessels which enter the wall of the buccal chamber, then two pairs of commissural vessels which link with a sub-pharyngeal cisterna of chambered

appearance. The dorsal vessel then passes through the walls of the "injector organ" to the prostomial coelom where it divides into two anterior lateral vessels which travel back through the "injector organ" wall, apparently between the two septal components, and continue as afferents to the sub-pharyngeal cisterna.

"Blind appendages" or, perhaps more correctly "blind capillaries", arise in large numbers from the anterior paired vessels, forming a dense plexus surrounding the pharynx and another within the prostomial coelom, as shown in Fig.1. Cardiac activity produces alternate swelling and deflating of the blind tips of the capillaries: at cardiac systole, 1.0 - 1.5mm of the tip swells to such an extent that the capillary wall stretches until it is virtually invisible under the low power of the microscope; at cardiac diastole, the tubular appearance is resumed.
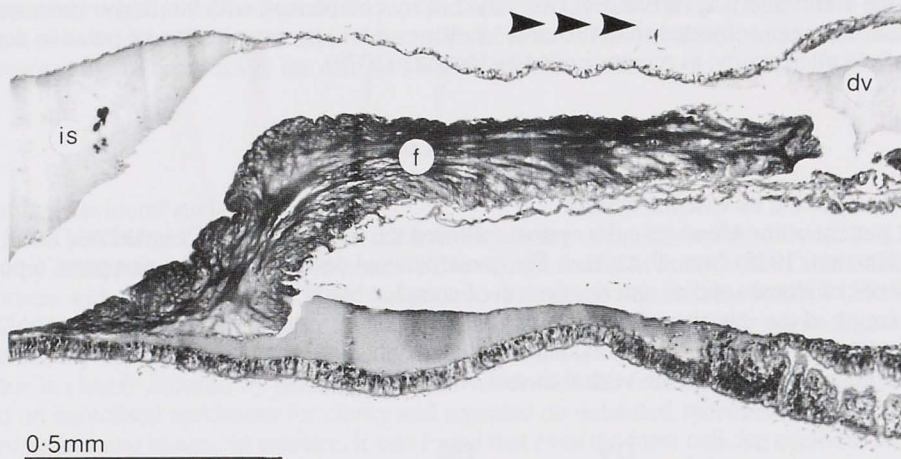


Fig. 2. — A median longitudinal section of the heart of *Ophelia bicornis*, - dv, dorsal blood vessel. - f, the flap valve. - is, intestinal sinus. The arrows point toward the anterior of the worm.

The increase in volume of the anterior blood system due to the simultaneous inflation of so large a number of blind capillaries is considerable. It seems that this activity is of benefit to *O.bicornis* in two ways.

The most obvious purpose of the mechanism is perhaps that of putting a large volume of blood, under pressure, in close contact with the coelomic fluid and its corpuscles. This may be an important means of producing coelomic fluid by an ultrafiltration process and of transporting dissolved molecules between the two body-fluid systems. This view gains some support from the presence of similar blind capillaries on the nephridial blood vessels of *O. bicornis* and from the experimental evidence, based upon amino acid uptake, which is presented below. Studies such as those of KOECHLIN (1966), NAKAO (1974) and RUPPERT & SMITH (1988) suggest that efficient internal fluid transfer in polychaetes is often effected under pressure across the thin walls of capillaries, sometimes organised into plexuses. Such efficiency may be achieved in *Ophelia bicornis* by means of the inflatable blind capillaries described here.

There is, however, a more spectacular purpose to this system which is of mechanical nature and easily observed as it occurs within the prostomial coelom. The "injector organ", similar to that described by CLAPARÈDE (1870) in *Ophelia radiata*, is a structure formed from the two remaining segmental septa, which seals off the prostomial coelom of *Ophelia bicornis* from the main body coelom. Observation of active *O. bicornis* shows that the "injector organ" plays an important rôle in the increasing of prostomial coelomic fluid pressure, but it is a passive rôle. The walls of the organ do not contract to any appreciable extent in *O. bicornis* and therefore little, if any, of its contained fluid is squeezed into the anterior chamber. The passivity of the "injector organ" and the relative inelasticity of the prostomial cuticle combine to make the prostomial coelom not only a sealed chamber, but also a relatively inexpandable one.

b. The uptake of dissolved amino acids.

HARRIS (1991b,c) has indicated that *O. bicornis* probably supplements its diet to a very considerable extent by the uptake of dissolved organic material from the interstitial water and from the surfaces of the sand grains which are crammed into the gut during the feeding cycle. Investigations using radioactively-labelled glutamic acid have

shown that the radioactive marker accumulates to a greater extent in the anterior part of *O. bicornis* than elsewhere, as shown in Fig. 3. Autoradiographic investigation of thin sections of the anterior region shows that a considerable proportion of the labelled amino acid accumulates in the coelomic corpuscles of the sealed anterior chamber, Fig. 4.
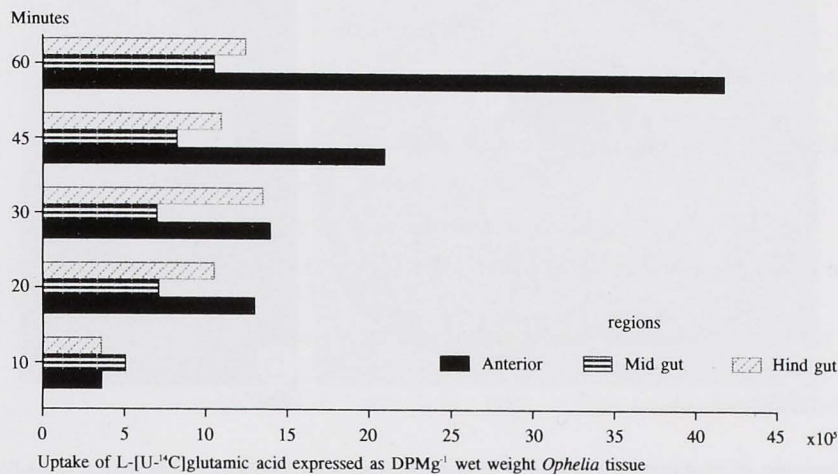


FIG. 3. — A bar chart showing typical uptake by *Ophelia bicornis* of radioactively-labelled glutamic acid over a sixty minute period. "Anterior" = the prostomium and buccal region, "Mid gut" = the central part of the body containing the absorptive part of the intestine, "Hind gut" = the rectum and pygidial papillae. DPM = disintegrations per minute in the scintillation counter.

## DISCUSSION

According to FAUVEL (1959) "L'appareil circulatoire des Sédentaires présente d'innombrables modifications en rapport avec la spécialisation des différentes régions du corps." Even within the context of such variety, the blood vascular system in the genus *Ophelia* is outstanding both in its structural modifications and in its functional relationships with the hydrostatic system and through both, with the behaviour and ecology of the worm. CLAPARÈDE (1870) described numerous blind vessels on the lateral vessels of *Ophelia radiata*: "Sur tout ce parcours le vaisseau dorsal et le vaisseau ventral sont mis en communication par une série d'anses. Le caractère le plus remarquable de cet appareil c'est que tous ces vaisseaux, surtout le dorsal et les anses, sont munis de centaines d'appendices aveugles, contractiles, dont le jeu alternatif de systole et de diastole est fort curieux à observer." He goes on to say that these structures are most numerous in the peripharyngeal region and are relatively rare within the prostomial coelom.

CLAPARÈDE (1870) described the "injector organ" as being of a tough, muscular nature and important to the generation of the prostomial rigidity which is so necessary for the penetration of sand. He said that this is accomplished by the contraction of the "appareil injecteur" by its own musculature, thus forcing into the enclosed prostomial coelom an additional volume of coelomic fluid, increasing the overall hydrostatic tension against the cuticle and so inducing a certain stiffness. In *O. bicornis* the "injector organ" seems to act only as the inelastic rear wall of the enclosed prostomial chamber.

At cardiac systole, the prostomial coelomic fluid is put under considerably increased pressure by the sudden and simultaneous inflation of the blind capillaries of the prostomial plexus. This pressure, developed against the inelastic cuticle, provides the necessary turgidity for the penetration of sand. At the same time, pressure is brought to bear on the anterior part of the pharynx which passes through the ventral wall of the "injector organ" and through the prostomial coelom and, due to the inflation of the plexus which lies external to the "injector organ", upon the posterior part of the pharynx also. This greatly facilitates the eversion, through the mouth, of the

voluminous pharynx tissues. It is probable that this pressure increase is supplemented by an increase in the volume of the pharyngeal coelomic fluid due to ultrafiltration through the greatly-expanded walls of the blind capillaries.
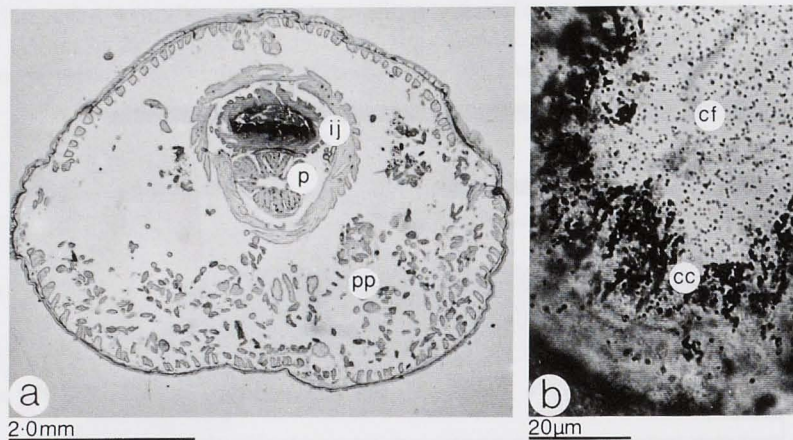


FIG. 4. — Autoradiographs of unstained sections of *Ophelia bicornis* after uptake of radioactive glutamic acid. a: a cross-section through the anterior pharyngeal region shows scattered concentrations of silver. b: part of the inner wall and coelom of the "injector organ" shows great concentration of silver in the coelomic corpuscles and scattered grains in the coelomic fluid. - cc, coelomic corpuscles. - cf, coelomic fluid. - ij, "injector organ". - p, pharynx. - pp, pharyngeal plexus.

HARRIS (1991a,c) described the typical habitat of *O. bicornis* in the Exe Estuary as being emersed for considerable periods, well — if not excessively — drained and meagre in potential food-sources and resistant to passage by a soft-bodied invertebrate. CHAPMAN and NEWELL (1947), having described the pressure which can be developed within the anterior coelom and proboscis of *Arenicola marina* state, suggested that this allows the worm to dig and to burrow effectively only when provided with thixotropic sand. It is not inappropriate to note, therefore, that the hydrostatic mechanism which is described here for *Ophelia bicornis* allows the worm to travel at all times with apparent ease through sand which is thixotropic for only relatively brief periods and dilatent for much longer ones.

The thinness of the gut-lining wall of the intestinal sinus of *O. bicornis* probably ensures the rapid transport of absorbed dissolved substances directly into the blood from the surfaces of the sand grains around which the walls of the gut wrap themselves in a totally-enveloping manner. This, coupled with the presence of a defaecation-enhancing device in the rectum (HARRIS, 1991b), gives an indication of the means which exist for efficient and constant nutrient uptake in this polychaete. Provision of a swift transport and storage mechanism, making use of an already-enhanced vascular system, would seem to be a logical ultimate development.

## CONCLUSION

The entire cycle of events may be summarised as follows:

a) *Ophelia bicornis* travels through highly resistant sand. Enhancement of the pressure within its prostomial hydrostatic skeleton by blood surges into expandable capillaries from a valved heart assists movement through the substrate.

b) Co-incidentally, the induced pressure system increases the efficiency of pharyngeal eversion and thus the intake of sand.

c) Nutrients absorbed from that sand are passed rapidly into the blood stream and efficiently flushed from the site of absorption to sites of temporary storage.

d) The same vascular apparatus permits rapid interchange of solutes between the blood and the coelomic fluid and its corpuscles which may act as one of the sites of food storage.

## ACKNOWLEDGEMENTS

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