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Spawning, Larval Development and Settlement Behaviour of *Gibbula cineraria* (Gastropoda: Prosobranchia) with a Reappraisal of Torsion in Gastropods

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Abstract

Cultures of developing larvae of *Gibbula cineraria* (L.) were obtained from adults spawning in the laboratory, and these cultures were reared to settlement of the larvae at 9 days. Dispersal of the outer jelly coat of the egg appeared to be delayed in the presence of spermatozoa. Early cleavage followed the typical spiral pattern, and gastrulation was by epiboly. The trochophore larvae hatched at about 28 h, before shell-formation began. The first 90° of torsion was completed between 48 and 56 h. The second part of torsion was completed within 4 days, and preliminary attempts to retract into the shell were made at 76 h. After 96 h, although torsion was complete, the larvae were still unable to retract fully into the shell. The larvae remained in a stage of "swimming-attempted creeping" until the 4th or 5th day after torsion was complete, but were unable to pull the shell upright until the end of this time. Throughout this stage, the velum was reduced and was finally shed. The animals all died after metamorphosis, which occurred at about 9 days, when the larval shell comprised $1^1/_2$ whorls, with a breadth of 250 to 300 μm. Settlement appeared to be passive, i.e., governed by wave action and tides. In the light of these observations, theories concerning torsion are reappraised. The advantage to the larva of torsion is not clear, as the larvae are not able to retract into the shell until after metamorphosis, yet it is clear from previous authors' observations that the first part of torsion in most gastropods involves only larval structures. This makes it difficult to suggest that torsion is entirely of advantage to the adult snails. It is thus proposed that the two components of torsion may have evolved independently. The first, relatively rapid component of torsion, resulting in 90° of displacement of the mantle cavity in relation to the visceral mass, may be of advantage to the swimming larva. The final, slower component of torsion may be of advantage to the newly-metamorphosed benthic snail, and is the only component of torsion found in those gastropods which have no free-swimming larva.

Introduction

The adults of *Gibbula cineraria* (L.) are dioecious and shed their gametes freely into the sea. Fertilization is external and the eggs and larvae are planktonic. According to Fretter and Graham (1962), the larval stages are unknown in the plankton, although the presence of eggs presumed to be those of *Gibbula* were reported to be present in the plankton at Plymouth by Lebour (1937). In *Calliostoma zizyphinum* (L.), however, the spawn is deposited in an egg-ribbon, and direct development occurs (Robert, 1902; Crofts, 1955). Apart from these observations, there have been few investigations of the early development and larval settlement of intertidal archaeogastropods.

Boutan (1885) described the development of *Fissurella* and later (1898, 1899) that of *Acmaea virginea* (L.). Patten (1886) described the development of *Patella* (L.), which has been further investigated by Smith (1935) and Dodd (1957). The development of a trochid (*Monodonta turbinata* Born) was first described by Robert (1899), and this was followed (Robert, 1902) by descriptions of the development of *Gibbula magus* (L.), *Calliostoma zizyphinum* and *Cantharidus striatus* (L.). Crofts (1937) described, in detail, muscle morphogenesis in the development of *Haliotis tuberculata* (L.), and later (Crofts, 1955) related these findings to the embryology of the other species described by earlier workers. Since Crofts' (1955) beautiful analysis of development, there have been accounts of the development of the American *Haliotis rufescens* Swainson (Carlisle, 1962), the Hawaiian trochid *Euchelus gemmatus* (Gould) (Duch, 1969), and the Red Sea species *Trochus erythraeus* Brocchi (Gohar and Eisawy, 1963). Anderson (1965) reared the larvae of three species of Australian Acmaeidae, and made observations on their settlement. The development of *Gibbula cineraria* was briefly mentioned, but not illustrated by Gersch (1936), and the only British trochid for which planktonic development has been described is *Monodonta lineata* (da Costa) the larvae of which were reared by Desai (1959, 1966). There have been few observations on behaviour during settlement, and only Desai (1966) has described the site of settlement of newly-metamorphosed young of any of the British trochids.

In conjunction with a study of the reproductive cycles of the common intertidal trochids (Underwood, 1971), it was decided to attempt to rear the larvae of those species with planktonic eggs, in order to carry out experiments on settlement preferences. Attempts to rear larvae from artificial fertilizations, and attempts to induce spawning in the adults in the laboratory failed. It did prove possible, however, to rear the larvae of *Gibbula cineraria* to settlement, and to make observations on their behaviour during settlement, although an experimental analysis of such behaviour was not carried out.

Material and Methods

Specimens of *Gibbula cineria* were collected at approximately monthly intervals throughout 1969, 1970 and 1971 from the shore at Heybrook Bay, Plymouth (England). The gastropods were maintained in flowing laboratory sea water at Bristol, with aerators but were unfed. They were usually used within a few days of collection. Numerous methods of treatment of preparations of gametes from adult individuals were tested in attempts to cause artificial activation or fertilization of the eggs (Underwood, 1971) but none of these methods proved reliable, and it was impossible to obtain viable cultures of developing larvae in the laboratory from artificial fertilizations, except when the gastropods were actively spawning.

In addition to this, various methods of treatment were tested throughout 1970 and 1971 in attempts to induce spawning in the laboratory (Underwood, 1971), but, although gametes were often released, fertilization and subsequent development of the eggs were never observed. By using individuals collected from various sites in Falmouth and from Heybrook Bay, it was found only in April, 1970 and 1971 that any or all of these methods caused ejaculation and spawning, provided the specimens were maintained at a temperature greater than 12 °C, in light. The gastropods were transferred to a laboratory at Falmouth, and maintained in tanks of static sea water. These were supplied with aerators and a 40 W lamp was placed over each tank. When the temperature of the water in the tanks reached 11° or 12 °C, the gastropods began to spawn.

Results

Spawning and Gametes

Usually males ejaculated first, but this was not always the case. In a tank with approximately 60 adults, almost invariably more than half spawned when the threshold temperature was reached. In both males and females, gametes were shed directly into the water through an upward fold of the right neck lobe of the epipodium. This formed what amounted to a closed tube, with the shell as the upper portion. Gametes were poured through this, in dense clouds of sperm, or bursts of 30 to 40 eggs. Spawning and ejaculation sometimes continued for several hours, during the course of which each female laid several hundred eggs. The same individuals could be made to spawn on consecutive days for 3 or 4 days. If cracked open after this, the ovary of the female was found to contain brown, spent patches, where no ripe ova were visible. This corresponded exactly with the condition of the ovaries of specimens collected at Heybrook Bay, Plymouth in May and June, 1971 and indicated that this was the time of year when spawning normally occurred on the shore (Underwood, 1971).

Contrary to the observations of Gersch (1936), animals often spawned in air, having previously crawled out of the water. In all cases where this was observed, the gastropods were on the vertical side of the tank and the gametes thus fell into the water.

Very large quantities of sperm were released in *Gibbula cineraria*, so that after approximately 1 h, the water became completely opaque. The water was not stirred, as agitation made collection of the eggs very difficult. In nearly all cases, however, normal development occurred, and there was no evidence of polyspermy.

Within a few minutes of release, the ova showed elevation of the outer layer of the gelatinous coat. Eggs released directly from the dissected ovary for artificial fertilization attempts showed exactly the same reaction, confirming the supposition of Fretter and Graham (1962) that both layers of the jelly around the ovum are formed in the ovary. After varying intervals (Table 1) this outer layer dispersed, leaving the rounded ova with a single layer of jelly, surrounded by the vitelline membrane. Fertilization could occur after this, whereas whilst the eggs were rounding off, numerous sperm became attached to the outer layer of jelly and none was ever seen to penetrate further. In the description of development in this paper, the time of fertilization was taken as the time of dispersal of the outer layer of jelly.

Table 1. *Gibbula cineraria. Time for dispersal of outer layer of jelly coat of ova at 12 °C (min from spawning)*

Culture	First of 10 ova	Last of 10 ova	Mean for 10 ova
In presence of sperm			
Stirred	30	45	37
Unstirred	45	70	54
In absence of sperm			
Stirred	20	45	32
Unstirred	20	45	30

This dispersal of jelly (Table 1) took longer in the presence of sperm than when the eggs were collected in sperm-free water. Gentle swirling of the eggs in a 500 ml beaker half full of water made no difference to dispersal in the absence of sperm, but greatly reduced the average time of dispersal in the presence of sperm. The significance of these observations was not clear, but probably the outer layer of jelly normally functions to prevent insemination of the eggs before they have finished the processes of rounding-off. Dispersal was postponed when sperm were present.

Eggs of *Gibbula cineraria* were approximately 200 μm in diameter, reaching a maximum of approximately 350 µm when the jelly coat was swollen. There was no evidence of a micropyle (contrary to the observations of Desai (1966) on *Monodonta lineata*), although the former stalk of attachment of the ovum in the ovary was clearly visible.

The sperm, which became active within a few moments after ejaculation, were typical diotocardian eupyrene sperm. They have been described by Franzén (1955), and were Type I sperm in the classification of Nishiwaki (1964). They were considered by him to have acrosomal adaptations for piercing the jelly coats around externally-fertilized eggs.

Acrosomal discharge could be brought about by the addition of egg-water to a very dense concentration of newly-ejaculated sperm. The acrosomal cap disappeared, leaving a thin filament anterior to the nucleus. Sperm motility was maintained for over 36 h after ejaculation, if in relatively large quantities of sea water.

Artificial fertilizations were performed during April, 1970 and 1971. These gave the normal pattern of cleavage (described below), but only in 1971 did larvae develop. Cultures of naturally spawned gametes from Falmouth were gently stirred continuously to prevent the eggs from settling on the bottom of the 500 ml beakers, as it was found that this reduced predation by infestations of ciliate Protozoa. The water in the cultures was changed twice daily, and was collected from a sandy beach and passed through coarse filter-paper.

Cleavage

Cultures were maintained at 12 °C and cleavage appeared to be typically spiral, as described in meticulous detail by Robert (1902) for *Gibbula magus*. Before polar-body formation, the germinal vesicle disappeared. The first polar body was extruded 20 to 45 min after fertilization and was followed at 40 to 90 min by the second one. They lay together at the animal pole, near the point of origin of the first cleavage furrow. The polar-bodies were visible inside the egg membrane until hatching of the trochophore.

The first cleavage furrow usually began to form within 15 min of the appearance of the second polar body (55 to 105 min after fertilization). The second cleavage was meridional, and was completed by 1.40 h. The 8-cell stage was completed by 2.30 h.

During the next division there was often asynchronous cleavage of the micromeres and the macromeres. Where this occurred, forming a transient 12-cell stage, the macromeres always divided first, giving rise to the second quartet of micromeres. The first quartet divided to give 16 cells, although this division often occurred before the 12-cell stage was complete. The 16-cell stage was usually complete after 3 h.

The subsequent cleavages were more rapid, and increasingly asynchronous. Thus, the fifth cleavage gave rise to transient 20, 24- and occasional 28-cell stages. The 20-cell stage was completed in 3.15 h. A transient 24-cell stage was often completed at 3.35 h as a result of the division of the second quartet of micromeres. This was followed by division of the other micromeres to give a 32-cell stage, which was completed at 4.30 h. The following divisions were of these micromeres, the central ones of which began to sink slightly below the surface at about 6.30 h (approximately 70 cells) but this never became a clear depression. Robert (1902) described the formation of an apical invagination in *Gibbula magus* which faded at about the 145-cell stage. In *G. cineraria*, no such invagination was observed.

Gastrulation and Trochophore

Gastrulation was, as described by Robert (1902) for *Gibbula magus*, by epiboly of the micromeres rapidly spreading downwards and enclosing the macromeres. The blastopore was wide at first, and gradually became constricted by 11 to 12 h. The trochoblasts became ciliated shortly after this, and by 16 h the prototroch was clearly visible towards the animal pole and the cilia were well developed (Fig. 1A). The ciliary beat was arhythmic. By this stage the blastopore was completely closed, after gradual displacement to a position just below the prototroch.

At 18 to 19 h, the ciliary beat became more regular and the trochophore larva began to rotate inside the egg membrane. At no stage in the development of the trochophore was there any sign of apical cilia, although the pretrochal cells were observed to be smaller than those of the posttrochal region. At 24 h, a degree of neural coordination had developed, so that the trochophore could stop and start the ciliary beat, whereas previously it had been continuous. The shell-gland invagination appeared at about 24 h, directly opposite the centre of the pretrochal area, not opposite the site of disappearance of the blastopore (as described for *Gibbula magus* by Robert, 1902, and for *Monodonta lineata* by Desai, 1966). Hatching occurred before the appearance of the shell, at about 28 h, and was accomplished by the gradual disintegration of the egg membrane, accompanied by violent movements of the prototrochal cilia.

Veliger and Torsion

Shell-formation began immediately after hatching, but appeared to differ from the process described by Desai (1966) for *Monodonta lineata*. Desai indicated that the rapid multiplication of the cells of the shell gland caused the eversion of that organ, as described for *Haliotis tuberculata* by Crofts (1937). Desai (1959) illustrated this stage as having numerous cells in the everted shell gland, standing clear from the main bulk of the larva. This was not described by Crofts (1937),

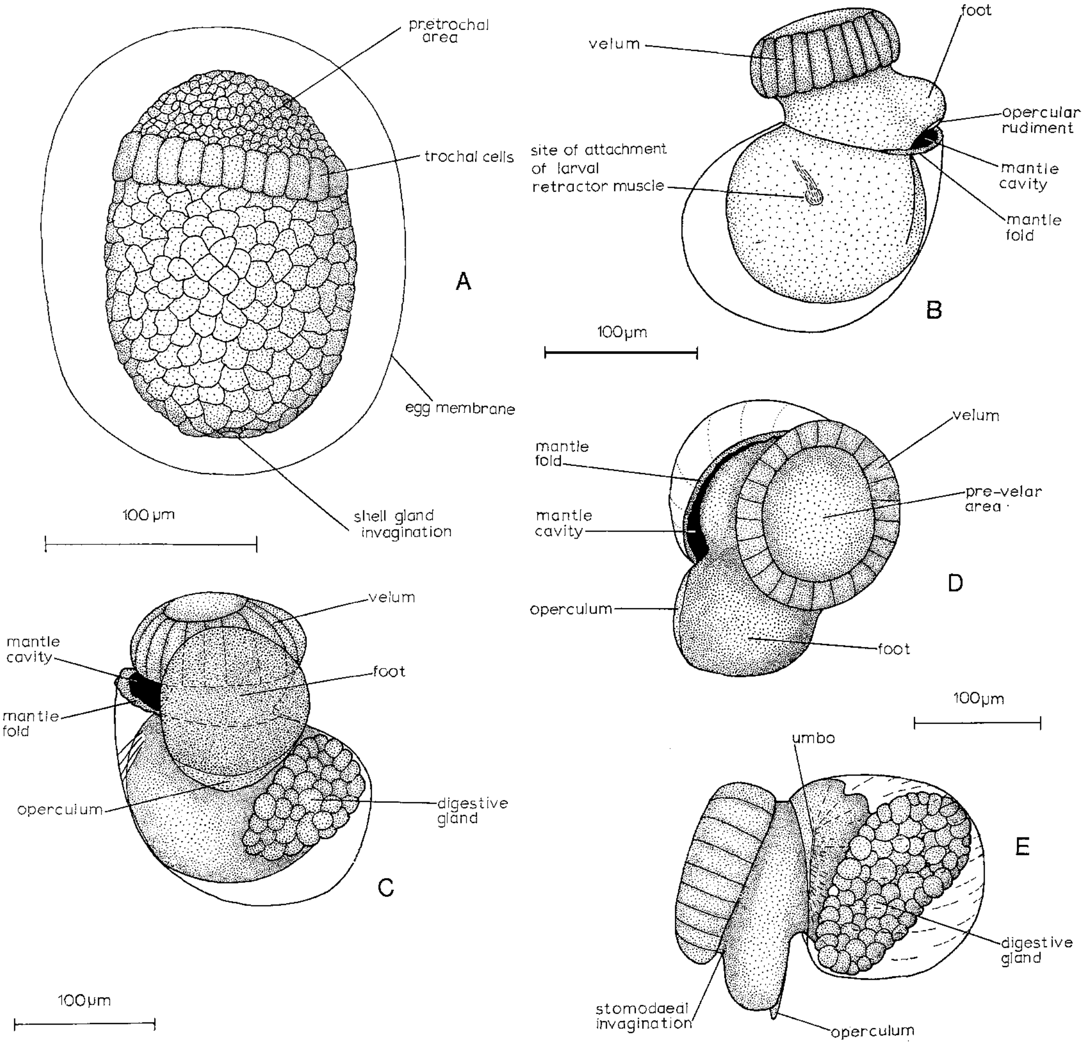

Fig. 1. *Gibbula cineraria*. Larva (locomotory cilia have been omitted). (A) Unhatched trochophore at approximately 26 h; (B) veliger larva from pretorsional right side at approximately 44 h; (C) (D) (E) veliger larva after the first 90° of torsion; (C) ventral view at approximately 56 h; (D) anterior view at approximately 70 h; (E) left lateral view at approximately 70 h

nor observed in the present study. Eversion of the shell gland occurred, resulting in a thin patch of cells on the surface of the trochophore. These gave rise to a transparent layer of shell, which gradually spread over the dorsal region of the larva, and then the shell-gland rudiment moved ventrally, giving rise to a cup-shaped transparent shell, which did not adhere to the body but was only in contact around its margin (Fig. 1B). At this time, the pedal rudiment was obvious (about 33 h) and was formed just below the point of closure of the blastopore. The pedal side of the gastropod was considered ventral in the pre-torsional veliger. The velar cells meanwhile migrated anteriorly, forming a distinct velum.

In *Gibbula cineraria*, the mantle fold and mantle cavity were first noticeable mid-ventrally. Robert (1902) described the origin of the mantle cavity as being mid-ventral in *G. magus*, but Crofts (1937) described it in *Haliotis tuberculata* as having an origin on the ventral right side of the larva. The mantle fold lay over the margin of the shell, posterior to the foot (Fig. 1B) and the mantle cavity was lined by mantle tissue. The operculum rudiment became visible at about 40 h, as a transparent patch on the posterior surface of the pedal rudiment. The digestive gland began to differentiate at approximately the same time, on the left side of the veliger. At this stage (40 to 44 h), immediately before the start of torsion, the point of attachment of the larval retractor muscle on the shell was easily seen, well forward on the right lateral face of the shell. This position was further forward than described by Crofts (1937) for *H. tuberculata* and very similar to the position in *Calliostoma zizyphinum* (Crofts, 1955). During this stage, the veligers swam near the surface of the water unless disturbed, when they would cease swimming and slowly sink. Even mild swirling of the water, however, would cause many of the larvae to become detached from their shells, when they would swim with the velum forwards, the foot downwards, and the detached larval retractor muscle trailing from the right dorsal region. This was of interest, as contraction of the muscle in the absence of the shell should presumably have pulled it over the dorsal surface of the larva and, thus, it should have appeared from the left dorsal region. This indicated that the larval retractor muscle had not yet begun to contract.

The first 90° of torsion took place between 48 and 56 h, presumably by contraction of the larval retractor muscle, as described by Crofts (1937, 1955). If the larval retractor muscle was looped from the pre-torsional right side over the dorsal surface as described by Crofts (1937, 1955) for *Haliotis tuberculata* and *Calliostoma zizyphinum*, it would have to cause more than 90° of torsion in order to straighten completely. Complete straightening of the muscle would result in the part of the pre-torsional left side of the foot to which it was attached coming to lie immediately ventral to the point of attachment on the right side of the pre-torsional shell (i.e., dorsal on the post-180° torsional shell). This form of displacement of more than 90° was illustrated by Crofts (1955) for *C. zizyphinum*. In *Gibbula cineraria*, however, the first part of torsion was completed within 8 h, and resulted in 90° of displacement, so that the mantle cavity lay on the right hand side, and the foot and velum had rotated, when viewed from the front, anti-clockwise in relation to the shell (Fig. 1C, D, E). The remaining part of torsion was completed within 4 days, the larva then being able, at least partially, to retract into the shell. The first phase of the second 90° of torsion was fairly rapid, and consisted of a gradual increase in the amount of rotation shown by the foot and velum in relation to the shell, so that by 70 h, the mantle cavity was latero-dorsal on the right side. The umbo of the shell was then somewhat ventrally displaced on the left side of the body. By 76 h, the umbo had moved even further ventrally, and the larva was beginning attempts to retract into the shell. Retraction of the mantle fold from the shell mouth preceded retraction of the velum and foot, as described for some other gastropods by Thompson (1967).

By 96 h the process of torsion was complete, but the larvae were never observed to retract into their shells. The most effective retraction even after disturbance, appeared to be ineffective as a defence mechanism against ciliate predators, as the operculum was left somewhat open and the velum and foot were always partially out of the shell. During the second half of torsion, the velum gradually became reduced in size and split ventrally to form two bands of velar cells running dorso-ventrally and meeting around the dorsal region of the front of the head. The mouth became obvious for the first time at about this stage, whereas formerly the stomodaeal invagination was never clear (Fig. 2A). The larvae spent long periods of time motionless on the bottom and could swim only in a jerky manner. They repeatedly made attempts to creep, but were not strong enough to pull the by now fully endogastric shell into an upright position. Mortality in cultures at this stage was often high.

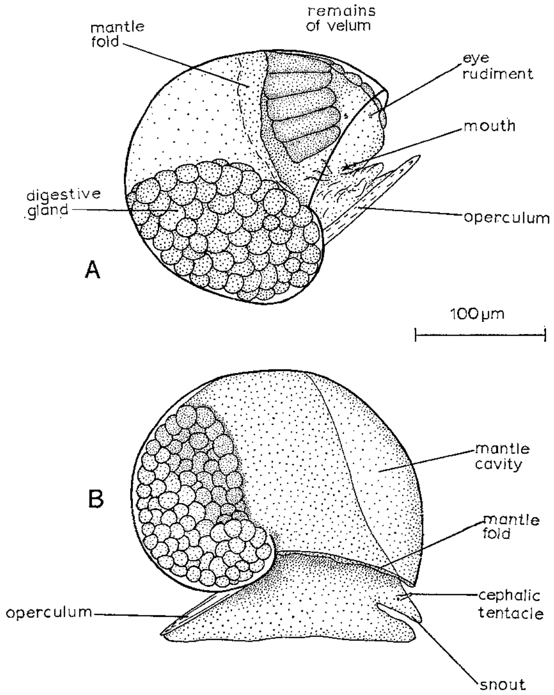

Fig. 2. *Gibbula cineraria*. Settlement and metamorphosis of larva. (A) 4-day larva after completion of torsion, attempting to retract into shell; right lateral view. (B) Crawling, benthic juvenile after shedding the velum; right lateral view

Many of the cultures had to be transferred to Bristol during this stage, and subsequent development may have been delayed by the change of conditions, although the cultures were maintained in Falmouth sea water at 12 °C. Mortality was no higher than observed in cultures which had passed through this stage in Falmouth.

Settlement and Metamorphosis

There was no specific time for the adoption of the benthic habitat; the larvae maintained the state of "swimming-attempted creeping" until the 8th or 9th day after fertilization, 4 or 5 days after torsion was complete. The post-veligers were then able to haul the shell upright and crawl with the sole of the foot adhering to the substratum. The velum throughout this period became considerably reduced, eventually remaining only as two bands of cells, with a few raggedly-beating cilia. These were of 4 or 5 cells only, on each side of the head. During metamorphosis, they were shed intact, not ingested as in many mesogastropod larvae (Fretter, 1969). This occurred when the shell was about $1\frac{1}{2}$ whorls, with a breadth of 250 to 300 μm. These corresponded in size to the smallest specimens of *Gibbula cineraria* found on the shore at Heybrook Bay, Plymouth during population studies (Underwood, 1971), and differed only in the degree of development of the tentacles and eyes.

During the last few days of post-veliger/premetamorphosis development, the eyes appeared in the pre-velar area. They first appeared as two pigmented patches, and gradually, as the eyes formed, the cephalic tentacles began to appear as outgrowths from the pre-velar surface. The mouth was definitely open and searching movements were made, especially as the snout developed. In none of the metamorphosed larvae was there any indication of the presence of a radula, either in observations of live individuals trying to feed, or in squash preparations examined microscopically. Anderson (1965) found no evidence for the start of feeding before settlement is permanent. The animals all died at this stage, i.e., at 9 days, after shedding the velum. Probably they were unable to feed, even though one culture was provided with *Chondrus crispus* as a potential substratum for feeding and source of diatoms. Less than 1% of all cultures reached this stage of development (Fig. 2B). No experiments were carried out on choice of substratum for settlement because of the length of time the larvae spent as post-veligers before crawling was established. During this inactive period, no means of locomotion would have been available to individuals on the shore, and they would have had to settle passively upon whatever substratum they became stranded as a result of waves and tidal action. This suggested that probably, in nature, the larvae could exercise no choice of substratum, and that intertidal juveniles would be found only in sheltered crevices or in algae (such as *C. crispus*) which offered some shelter.

Discussion

Settlement of Larvae

The major features of the development of *Gibbula cineraria* were described in the present study, from the external appearance of the embryos and larvae. They confirm, however, that development in this species follows the pattern for other trochids described by Robert (1902) and for other archaeogastropods described by Crofts (1937, 1955).

The facts that successful metamorphosis occurred in the absence of any natural substratum, and that a considerable length of time was spent in a non-motile state during settlement, indicated that there was probably little or no choice exercised in settlement behaviour. The larvae probably settle anywhere, and remain there if passive movement by waves and tidal action does not dislodge them. This would make sublittoral settlement most likely. Desai (1966) reported that creeping was well developed within 2 days in *Monodonta lineata*, and the juveniles were found only in the upper part of the shore. Unpublished observations (Underwood, 1971) also indicated that juveniles of *M. lineata* were found only in the upper part of the adults' zone, which indicated that, in this species, some influence on settlement was provided by the substratum in different parts of the habitat.

Investigations of other gastropods have shown that many species have highly developed mechanisms for choosing favourable substrata for settlement and metamorphosis. An ability to delay metamorphosis until a suitable substratum is reached, combined with a chemotactic ability to recognize suitable substrata, was shown by *Nassarius obsoletus* (Say) (Scheltema, 1961). Even more specific mechanisms have been shown in nudibranch larvae (Thompson, 1958, 1962), where the substratum chosen by the larvae of each species was the natural food of the adults. It is possible that the absence of a suitable substratum in the present observations delayed metamorphosis after the start of the adoption of the benthic swimming-attempted creeping habit, but this was unlikely, as the gastropods at this stage had no means of locomotion and their movements and position would be entirely due to water movements outside their control. There does, however, seem to be a very vulnerable period in the life history of the larvae, when swimming is no longer possible because of the increase in size of the larva and decrease in size of the velum. At the same time, crawling has not yet developed, possibly because the columellar muscle is not sufficiently developed to pull the shell upright.

Torsion

The relationship between torsion and retraction into the larval shell is somewhat obscured in the present observations by the inability of the larvae of *Gibbula cineraria* to retract fully into the shell at any stage of their development. Yonge (1947) brought together for the first time the numerous theories concerning torsion. This subject has since been reviewed extensively (Eales, 1950; Crofts, 1955; Morton, 1958; Ghiselin, 1966; Thompson, 1967). The majority of theories of torsion fall into two categories, those which attempt to explain the evolution of the process of torsion as an adaptation to the advantage of the larva, and those which consider it to be a necessary adaptation for the adult.

One of the major advantages supposed to accrue to the planktonic veliger as the result of torsion is that it enables the larva to retract head-first into the shell, leaving the least delicate of all its parts, the foot, until last. The operculum then seals the opening of the shell. This was considered by Garstang (1928), Yonge (1947), Eales (1950) and Crofts (1955) to be all that was necessary to explain the functional advantage of torsion and, therefore, its evolutionary origin. There is, however, some evidence to discredit this argument. Thompson (1967) showed that the gastropod larva does not retract into the mantle cavity at all, but retracts the mantle as well as the cephalo-pedal mass. This was confirmed for *Gibbula cineraria* in the present study, during its phase of partial retraction and, in fact, was illustrated (Fig. 17, Plate 23) by Crofts (1937) for *Haliotis tuberculata*. Thus, the retracted larva is inside the shell cavity, and there would appear to be no reason why this should not be possible even if the mantle cavity were posterior to the foot. In addition, Thompson (1962) observed that the larvae of the nudibranch *Tritonia hombergi* Cuv. could fully retract into the shell, even though torsion through 180° did not occur. Retraction of this species was, thus, possible while the deepest part of the mantle cavity was in a lateral position on the right-hand side of the larvae. Finally, in the present study, the larvae of *G. cineraria* were never seen to retract fully into the shell, and possibly never do in these early stages. This failure to retract into the shell was also observed in Australian species of Acmaeidae (Anderson, 1965). These observations indicate that retraction into the shell may not occur in some gastropod larvae and, where it does occur, can be carried out regardless of 180° of torsion.

There are, however, difficulties in the consideration of the evidence for the supposed evolution of torsion in gastropod adults. Ontogenetic torsion is entirely a larval feature, involving larval muscles which may have atrophied by the time of settlement and metamorphosis (Crofts, 1937, 1955). Therefore, it is unlikely that torsion arose first in adult ancestral molluscs.

Ghiselin (1966) suggested that torsion may have been an evolutionary adaptation which arose in the larva of a limpet-like ancestral mollusc, and was advantageous to the newly-settled larva in helping it to balance the shell during settlement. During the present study it was observed that the post-veligers of *Gibbula cineraria* were unable to crawl successfully even 4 days after torsion was completed. There was similar difficulty in *Haliotis tuberculata* (Crofts, 1937) and *Acmaea testudinalis* (Kessel, 1964). Anderson (1965) found that, in *Notoacmaea petterdi* (Ten.-Woods) and *Chiazacmaea flammea* (Quoy and Gaimard), crawling was very feeble, and that the larvae were very unstable during the days immediately after settlement. Ghiselin (1966) used these observations on the difficulty of shell balance in newly settled, post-torsional larvae as evidence for the advantages of torsion to this stage in the ontogeny of the animals. The difficulties in locomotion experienced by newly-settled *G. cineraria* seem to be because of the relatively late development of an adequate columellar muscle rather than a lack of balance or displacement of components of the body.

Perhaps then, the two components of torsion arose independently, with selective advantages for the different ontogenetic stages. The first, relatively rapid, component of torsion, causing approximately 90° displacement of the mantle cavity by contraction of the larval retractor muscle, may have evolved in the larva to suit planktonic needs. The remaining component of torsion, brought about by differential growth of different parts of the shell, with displacement of the point of attachment of the larval retractor muscle, may have arisen at or after settlement, as in *Haliotis tuberculata* during the early benthic stage (Crofts, 1937) as an adaptation to suit the needs of the adult.

The second part of torsion often does not occur until after the planktonic stage is over (as in *Haliotis tuberculata*) or not at all (as in nudibranchs); the latter do show the initial part during their planktonic larval stage (Thompson, 1962). The advantage of 90° torsion to shelled planktonic larvae must, however, be explained. Garstang's (1928) theory does not seem acceptable (Thompson, 1967, see above). Instead of being defensive, the advantage of this first part of torsion may be in the balance and relative position of the velum in relation to the bulk of the shell. If the larvae swam only with the velum upwards, this conclusion would be dubious, as the position of the shell would be irrelevant. The veligers, do, however, also swim directionally, in which case there may be hydrodynamic advantages to the larva in alteration of the position of the visceral mass in relation to the direction of movement.

Some evidence for the hypothesis that the first 90° of torsion evolved entirely as a larval adaptation is provided by those extremely specialized prosobranchs which have no free-swimming veliger larvae. In several such species of prosobranch, the whole 180° process of torsion is by slow differential growth, and no larval muscles develop before torsion is complete. This is found in *Viviparus* (L.) (Drummond, 1902), *Pomatias elegans* (Müller) (Creek, 1951), *Littorina littoralis* (L.) (Delsman, 1914), *Crepidula* (L.) (Conklin, 1897), and the marine whelk *Ocenebra aciculata* L. (Franc, 1940) none of which has free-swimming larvae. The much more primitive archaeogastropod *Calliostoma zizyphinum* also has no free-swimming larvae, but torsion is not all by differential growth (Crofts, 1955). *C. zizyphinum*, however, also retains other primitive features, e.g. the prototroch, which is lacking in *P. elegans*. To clarify the relationship between the lack of larval retractor muscle morphogenesis in torsion and the lack of free-swimming larvae, further investigation of torsion is desirable, particularly in species which lack free larvae [e.g. *Littorina saxatilis* (Olivi)].

There is, thus, a correlation between the lack of larval retractor muscle morphogenesis in torsion, and lack of free-swimming larvae. This, coupled with the undoubted presence of 180° of torsion in all prosobranchs, indicates that the two components of torsion are separate and that the latter part is necessary for the adult, regardless of the presence or absence of free larvae in its ontogeny. The advantage of torsion to the adult may be the closer juxtaposition of the inhalent current (in aquatic forms) to the head and sense organs (as proposed by Morton, 1958). Alternatively, it may reside in advantages in locomotion and/or respiration, the weight of the shell not being thrown onto the pallial cavity as a result of 180° torsion (Naef, 1911).

Summary

1. Cultures of developing larvae of *Gibbula cineraria* (L.) were reared to settlement of the larvae at 9 days.

2. Dispersal of the outer jelly coat of the egg appeared to be delayed in the presence of spermatozoa, and probably this outer layer of the jelly prevents polyspermy.

3. Early cleavage was typically spiral, and gastrulation was by epiboly. The trochophore larva hatched at 28 h, before shell formation began. The first 90° of torsion was completed between 48 and 56 h. The slower second part was completed within 4 days. Preliminary attempts were made to retract into the shell at 76 h, but complete retraction of the larvae was never observed.

4. The larvae remained in a state of "swimming-attempted creeping" until the 4th or 5th day after the completion of torsion, but were unable to pull the shell upright until the end of this time. The velum was reduced throughout this stage and was finally shed. Settlement appeared to be passive (i.e., governed by waves and tides) as the larvae were unable to move very actively when they first settled.

5. Theories concerning torsion are discussed. It is proposed that the two components of torsion may have evolved independently. The first, relatively rapid component of torsion, resulting in 90° of displacement of the mantle cavity in relation to the visceral mass, may be of advantage to the swimming larva. The final, slower component of torsion may be of advantage to the newly-metamorphosed benthic snail, as it is the only component of torsion found in those gastropods which have no free-swimming larva.

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