



Second Summer Course in Embryology  
of Marine Invertebrates  
13 June - 3 July 2016

# The White Sea Invertebrates Development



WSBS MSU  
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**This is a short atlas illustrating the development of the most common species of the White Sea invertebrates. The atlas has been prepared for the Second Summer Course in Embryology of Marine Invertebrates, 13 June – 3 July 2016, the White Sea Biological Station of Lomonosov Moscow State University (WSBS MSU).**

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## PORIFERA

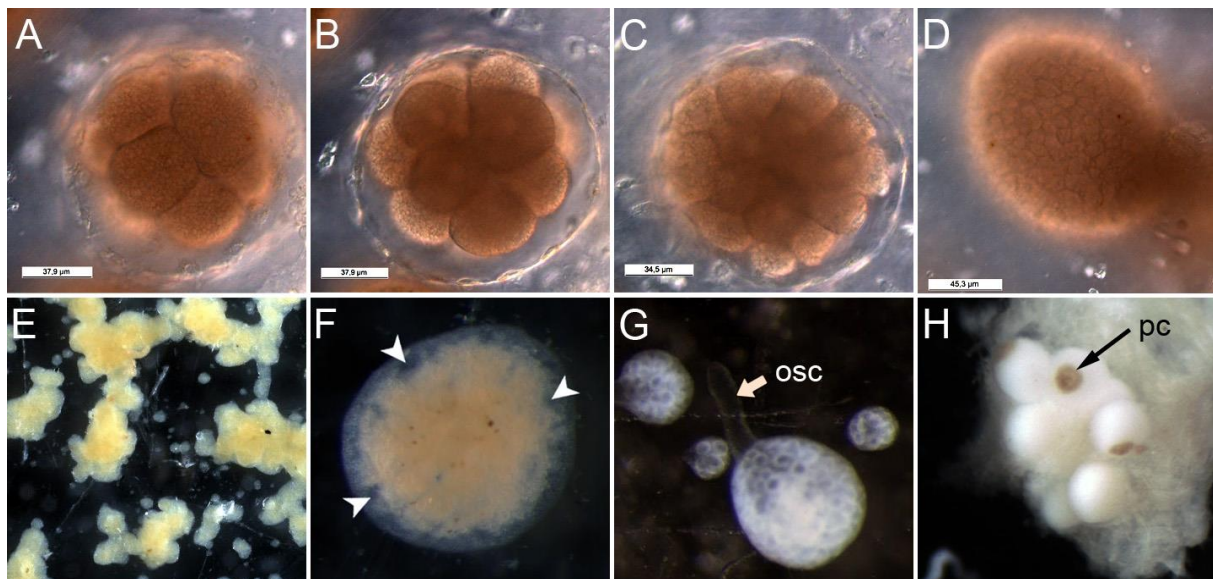
Several species of sponges are available for development study at the beginning of summer. One of them, *Halisarca dujardini* (Johnston, 1842), is common in rhizoids of brown algae and has an irregular body shape with a smooth mucous surface. There are many matured oocytes inside the sponge at the end of June. Using dissecting needles, individual embryos can be found at various developmental stages. After uniform holoblastic cleavage (Fig. 2 A-C), the blastula is formed (Fig. 2 D). Different larvae types are formed at the next stage – parenchimulae, disphaerulae or coeloblastulae. Larvae leave the maternal sponge at the beginning of July. *Halisarca* is able to regenerate its entire body from dissociated cells. The cells aggregate and form primmorphs during a 24 h period following dissociation (Fig. 2 E) and an aquiferous system is formed (Fig. 2 F, G) during the next several days.

*Haliclona aquaeductus* (Schmidt, 1862) has a pulvinate body with crater-shaped osculumes on the surface (Fig. 1). In June, embryos at different stages can be found in the basal parts of sponges. Generally, there are white parenchimulae with a ring of brown pigmented cells around the posterior pole (Fig. 2 H).



**Figure 1. *Haliclona aquaeductus*.**

Several osculumes (osc) are on the surface of sponge.



**Figure 2. Development of White Sea sponges.**

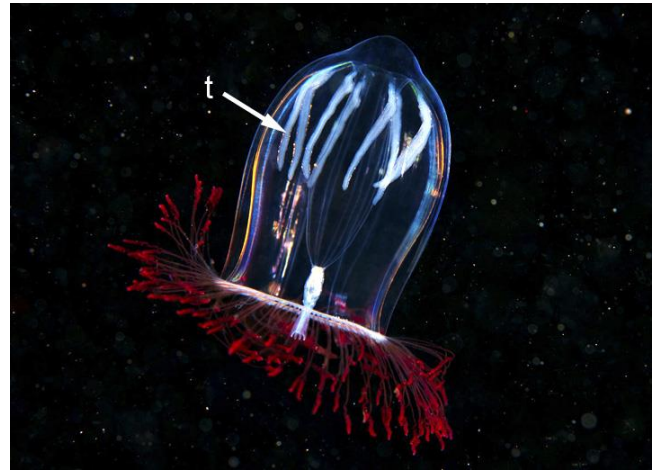
**A-D** – *Halisarca dujardini* embryonic development; **A-C** – cleavage embryos; **D** – blastula; **E-G** – reaggregation of dissociated *H. dujardini*; **E** – cell aggregates 2 h after dissociation; **F** – attached primmorph with formed aquiferous system cavities (arrowheads); **G** – regenerated sponge with formed osculum (arrow); **H** – *Haliclona aquaeductus* parenchimulae, pc – pigmented cells.



**CNIDARIA, HYDROZOA – *Aglantha digitale***

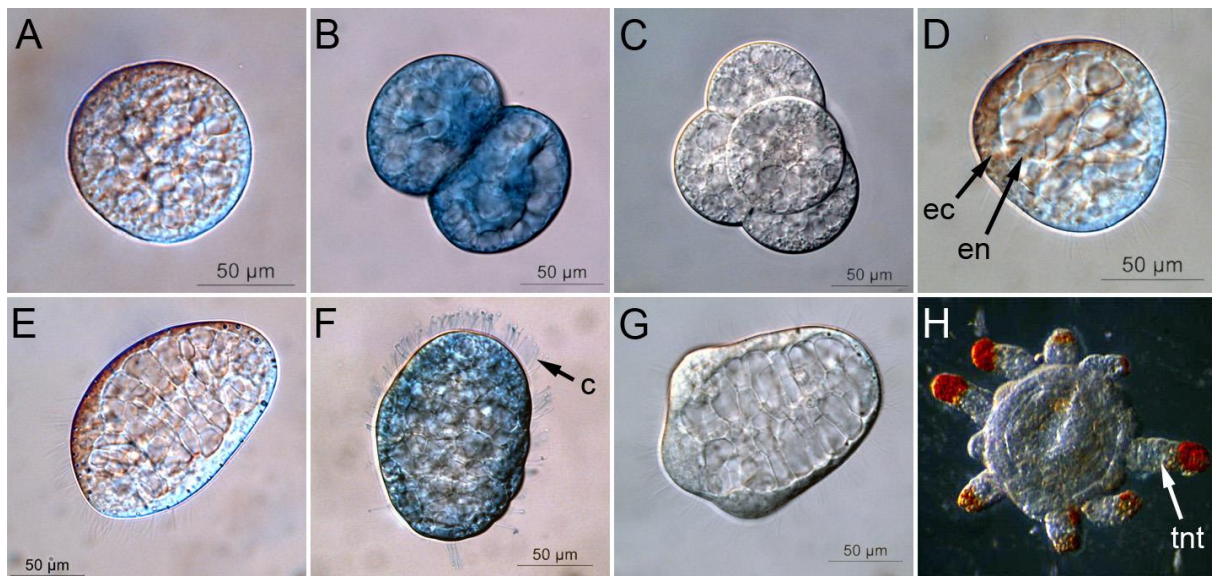
Like other members of the order Trachymedusae, *Aglantha digitale* (O.F. Muller, 1776) has no polyp stage in its life cycle. Adult jellyfishes have an elongated (about 1-1.5 cm) transparent bell with many tentacles around the edge (Fig. 3). Eight elongated gonads are located in the upper part of the subumbrella cavity. *Aglantha* jellyfishes are dioecious; males can be recognized by the homogeneous structure of the gonads contents. In the White Sea, their reproduction takes place from mid-June to August. Mature gametes are spawned into the water. Following the incubation of males and females in separate containers with filtered seawater, mature gametes can be collected and in vitro fertilization can be performed.

Eggs of *A. digitale* (Fig. 4 A) are rich in yolk, undergo complete cleavage (Fig. 4 B, C) and form morula. Then, the separation of the ectoderm and endoderm by delamination takes place (Fig. 4 D). A planula covered with cilia (Fig. 4 E, F) does not settle, but forms at first an actinula-like larva (Fig. 4 G, H) and then a young medusa.



**Figure 3. Adult male of *Aglantha digitale*.**

Eight testes (t) are visible in the upper part of the bell.



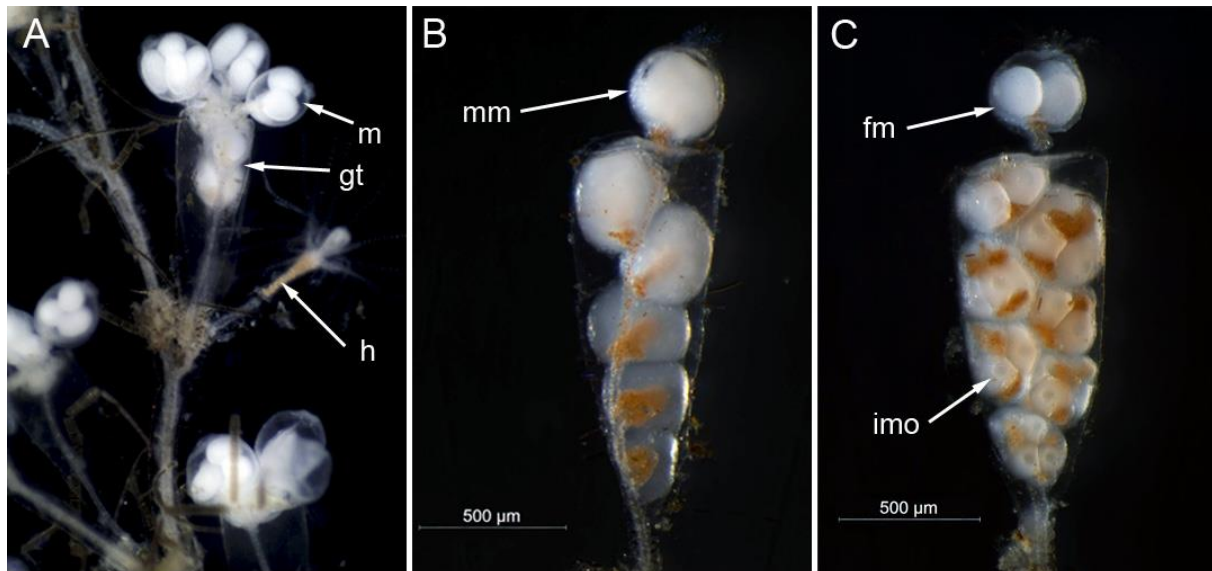
**Figure 4. Development of *Aglantha digitale*.**

**A** – zygote; **B** – 2-cell embryo; **C** – 4-cell embryo, which formed a tetrahedral structure; **D** – gastrulation due to delamination, ec – ectoderm, en - entoderm; **E** – planula; **F** – planula stained with Janus Green, cilia (c) are visible; **G** – specific rocket-like late planula; **H** – actinula-like larva with tentacles (tnt) which later develops into a medusa.

**CNIDARIA, HYDROZOA – *Gonothyrea loveni***

Colonies of *Gonothyrea loveni* (Allman, 1859) form low and dense (1-3 cm) thickets on brown algae in the lower tidal zone and upper subtidal zone. Reproduction takes place in June-July, when gonothecae with maturing medusoids appear in colonies (Fig. 5 A). Mature medusoids leave the gonothecae, but remain attached to it (Fig. 5 A-C). Colonies of *G. loveni* are dioecious; 2-4 eggs mature in every female medusoid (Fig. 5 A, C). Fertilization and embryonic development occurs inside the female medusoid.

To observe embryonic development, an individual embryo needs to be removed from the medusoids using dissecting needles. Note that every medusoid contains embryos at the same stage of development.

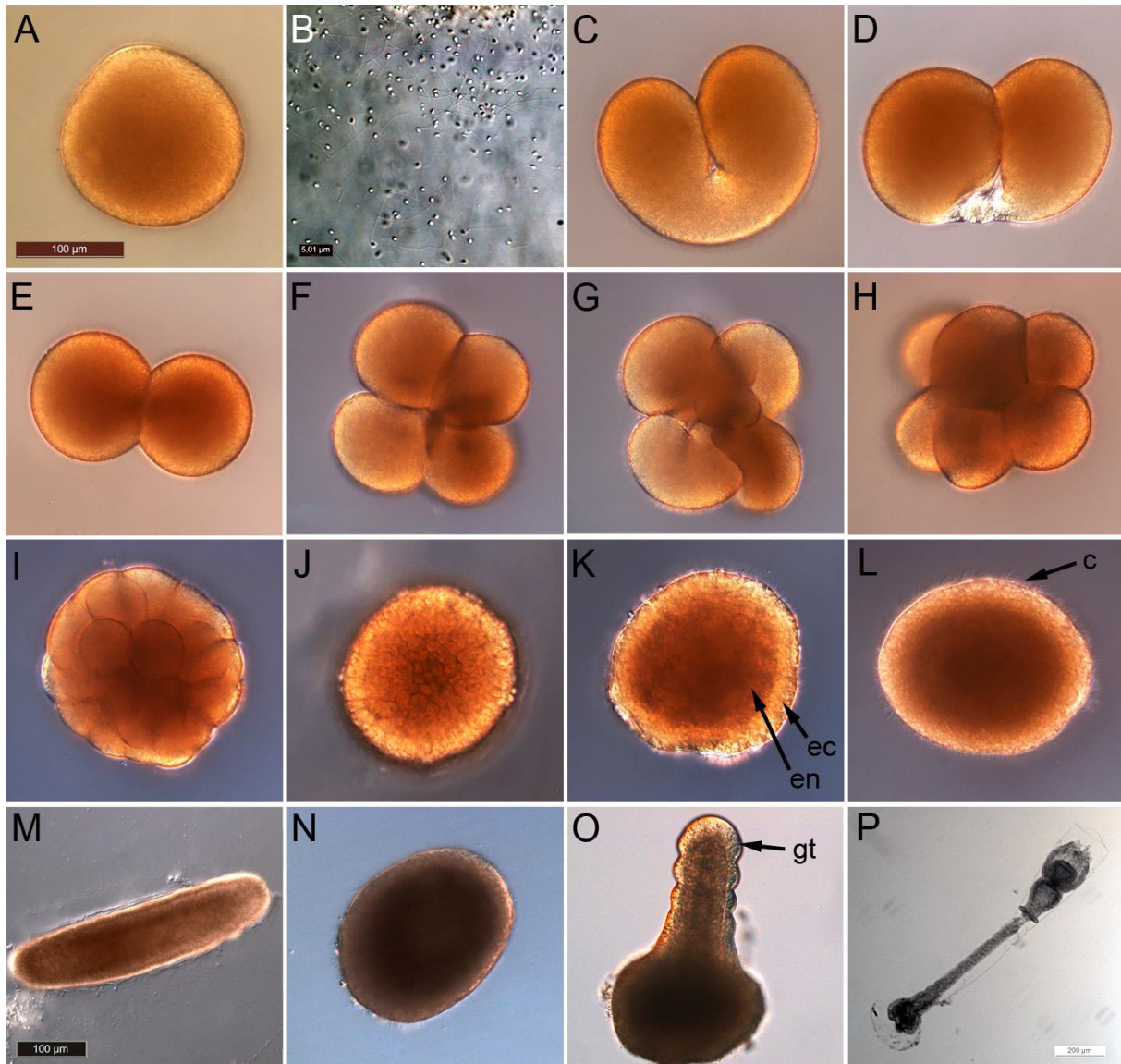


**Figure 5. Colonies of *Gonothyrea loveni*.**

**A** – Colony fragment with hydrant (h) and female gonothecae (gt) with excurved mature medusoids (m); **B** – the male gonotheca with excurved mature male medusoid (mm); **C** – the female gonotheca with excurved mature male medusoid (fm) and containing the medusoids with immature oocytes (imo).

*Gonothyrea* exhibit a specific type of cell division called unilateral cleavage. This means that a cleavage furrow forms at one pole of the cell and progresses to the other pole, so that cells appear heart-shaped in mid-cleavage (Fig. 6 C, G). It is impossible to trace any regularity in the directions of cleavage divisions; thus, cleavage of *G. loveni*, as well as most other hydroids, is anarchistic in terms of type. Later during development, morula formation (Fig. 6 I-J) and gastrulation due to delamination (Fig. 6 K) take place.

The embryo develops into a planula larva (Fig. 6 M) that leaves the medusoid. The planula larva is characteristic for most cnidarians. The hydrozoan planula is uniformly ciliated, oval-shaped, usually somewhat opaque and lacks any appendages or gut (they do not feed). Hydrozoan planulae usually spend a short period of time in plankton (days), then settle (Fig. 6 N) and undergo metamorphosis into a benthic polyp stage (Fig. 6 O, P), the asexual generation in the life cycle of a hydrozoan. Planulae kept in a Petri dish with seawater settle in 1-2 days.



**Figure 6. Embryonic development and metamorphosis of *Gonothyrea loveni*.**

**A** – zygote; **B** – sperm cells derived from male medusoid; **C** – the first unilateral cleavage furrow; **D** – end of the first cleavage division; **E** – 2-cell embryo; **F** – 4-cell embryo; **G** – the third cleavage, unilateral cleavage furrows are visible; **H** – 8-cell embryo with pseudo-spiral cleavage pattern; **I** – early morula; **J** – late morula; **K** – gastrula, ectoderm (ec) and entoderm (en) are distinguishable; **L** – preplanula, forming cilia (c) are visible; **M** – planula; **N** – settled planula; **O** – formation of growing tip (gt) of primary polyp; **P** – formed primary polyp.



## CNIDARIA

### HYDROZOA

Many hydroids undergo metagenesis (alternation of generations) in their life cycle. Sedentary asexual polyp generation alternates with free-floating medusa generation that reproduces sexually. However, the reduction of life cycle stages (hypogenesis) occurs quite frequently. *Aglantha digitale* is one such example; it has no polyp stage. At the same time, *Gonothyrea loveni* medusa generation is reduced to attached medusoids. Unlike medusae (Fig. 7 A), medusoids have no mouth opening, but there are radial channels and a closed subumbrella cavity (Fig. 7 B). *Coryne lovenii* (M. Sars, 1846) has the same level of metagenesis reduction.

*Clava multicornis* (Forskal, 1775) forms procumbent colonies of bundles of the hydrants. Tight coupling of gonophores is formed on the hydrants, under the tentacles (Fig. 7 E). The rudiment of the subumbrella cavity is the only remaining medusoid sign (Fig. 7 C). The planula larvae are formed in the female gonophores in August. Gonophores are also formed on the hydrants of *Ectopleura (Tubularia) larynx* (Ellis & Solander, 1786). Actinula larvae with tentacles are formed in gonophores in July and August.

The next level of metagenesis reduction is observed in *Dynamena pumila* (Linnaeus, 1758) and *Laomedea flexuosa* (Alder, 1857). Gonangiums with sporosacs mature in the colonies during July (Fig. 7 F, G). Sporosacs are two-layer protrusions of the body wall (Fig. 7 D).

Many White Sea hydroids have a complete life cycle, with a free-floating medusa stage. A lot of small flat medusae (Fig. 7 H) of *Obelia longissima* (Pallas, 1766) appear in plankton in June and *Obelia geniculata* (Linnaeus, 1758) in August. Four gonads are arranged along the margin of the bell. Medusa generation of *Sarsia tubulosa* (M. Sars, 1835) (Fig. 7 I), *Bougainvillia superciliaris* (Agassiz, 1849) (Fig. 7 J) and *Rathkea octopunctata* (M. Sars, 1835) are common in plankton in June.

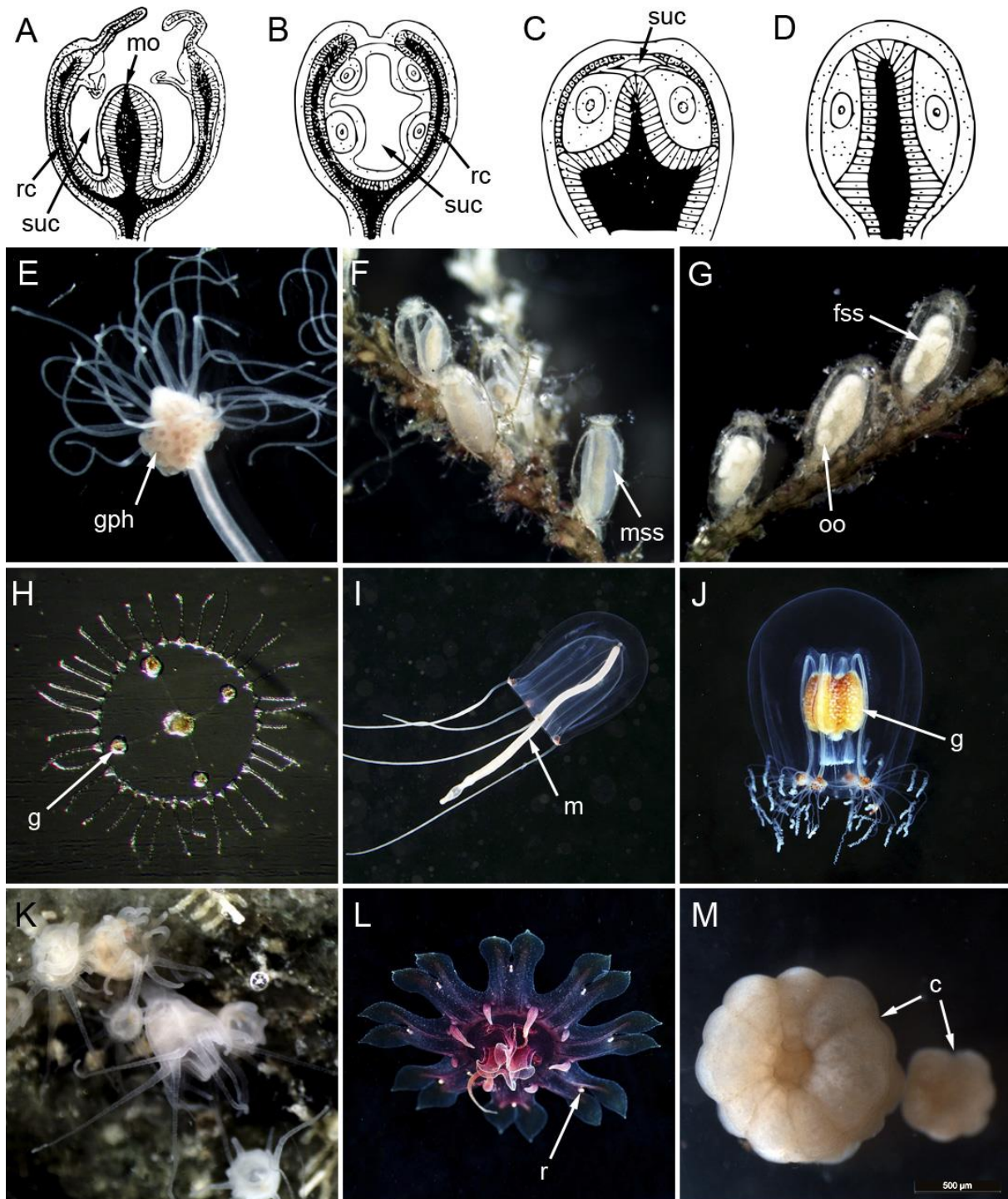
### SCYPHOZOA

*Aurelia aurita* (Linnaeus, 1758), *Cyanea capillata* (Linnaeus, 1758) and *Cyanea tzetlinii* (Kolbasova & Neretina, 2015) have metagenetic life cycles. Jellyfish are dioecious; fertilization and development take place in females' stomachs. Scyphistoma is a polyp stage (Fig 7 K) formed from the settled planula that reproduces by budding. The ephyrae (Fig. 7 L) are formed during strobilation of scyphistomae, frequently occur in plankton in June and develop into adult jellyfish for the entire summer.

### ANTHOZOA

Development of sea anemones *Aulactinia stella* (Verrill, 1864) is direct and occurs in the mother's body. Using genetic analyses, Bocharova and Mugue (2012) showed that juveniles can also be bred in the non-maternal organism. Juveniles (Fig. 7 M) with developing septae can easily be found in the gastric cavity of adult anemones.





**Figure 7. Development of White Sea cnidarians.**

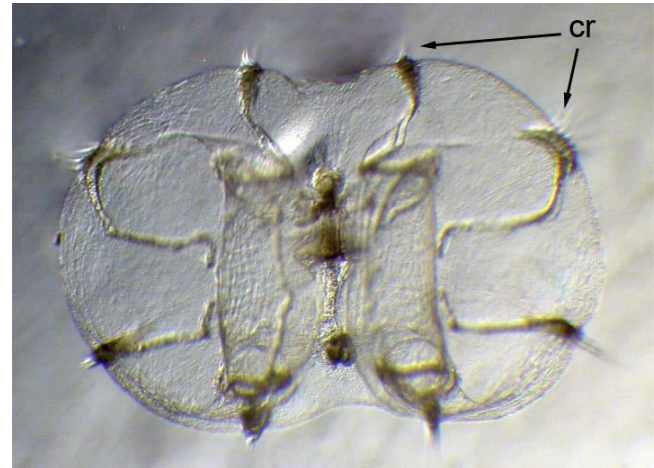
**A-D** – stages of reduction of medusa generation (Kuhn, 1913): medusa (**A**), medusoid (**B**), gonophore (**C**), sporosac (**D**), mo – mouth, rc – radial channels, suc – subumbrella cavity; **E** – *Clava multicornis* with gonophores (gph); **F** – gonangia of *Dynamena pumila* with male sporosacs (mss); **G** – gonangia of *D. pumila* with female sporosacs (fss), oocytes (oo) are visible; **H** – *Obelia longissima* medusa with four gonads (g); **I** – *Sarsia tubulosa* medusa, long manubrium (m) with gonads; **J** – *Bougainvillia superciliaris* medusa, manubrium with orange gonads (g) are visible; **K** – sciphistomae; **L** – *Aurelia aurita* ephyra, each of the eight lobes contains rhopalium (r); **M** – *Aulactinia stella* juveniles from the parent anemone, constrictions (c) coincide with developing septa.

**CTENOPHORA**

Several species of comb jellies can be found in the White Sea, including *Bolinopsis infundibulum* (O.F.Muller, 1776) and *Beroe cucumis* (Fabricius, 1780).

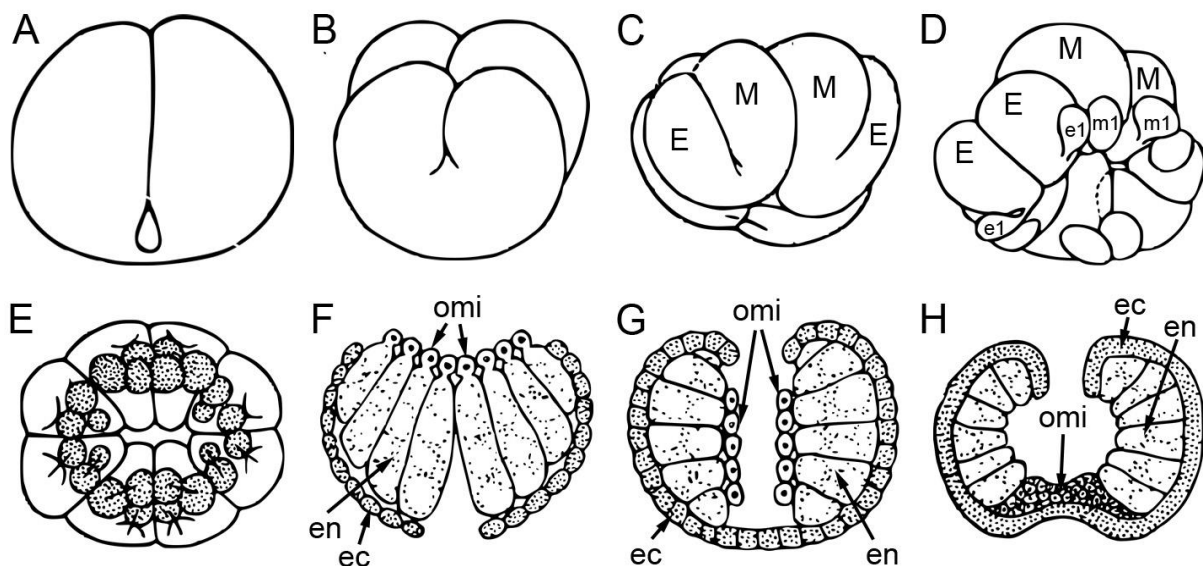
The embryonic development of all ctenophores proceeds extremely uniformly. Cleavage occurs by the forming of unilateral furrows (Fig. 9 A). The first and second cleavage furrows (Fig. 9 B) divide the egg according to the sagittal (pharyngeal) and tentacular planes of developing comb jelly. After the third cleavage, the embryo is divided into four central blastomeres M, and four lateral blastomeres E (Fig. 9 C). The next cleavage divisions are extremely unequal and divide embryos into large oral macromeres M and E, and small aboral micromeres m1-2 and e1-3 (Fig. 9 D). Micromeres form a cell layer (ectoderm)

that surrounds macromeres (entoderm) (Fig. 9 E-G). This process can be regarded as gastrulation by epiboly. Then macromeres are divided equally into 16 cells; 24 oral micromeres are formed and penetrate deeply into the gastric cavity (Fig. 9 F, G), where they form cross-shaped primordium of tentacles muscles (Fig. 9 H). This structure can be regarded as mesodermal. Cydippid larva with eight comb rows (Fig. 8) forms and hatches then.



**Figure 8. Ctenophora juvenile.**

View from the oral end. Eight comb rows (cr) are visible.



**Figure 9. Embryonic development of ctenophores.**

**A-E** – cleavage of *Beroe ovata* (Ziegler, 1898); **A** – the first cleavage division by unilateral furrow formation; **B** – the second cleavage division; **C** – the third cleavage division, M and E blastomeres are formed; **D** – the fourth cleavage division, micromeres m1 and e1 are formed; **E** – cleavage embryo, aboral view; **F-H** – germ layers differentiation (Davidov, 1914), lateral view, ec – ectoderm, en – entoderm, omi – oral micromeres.

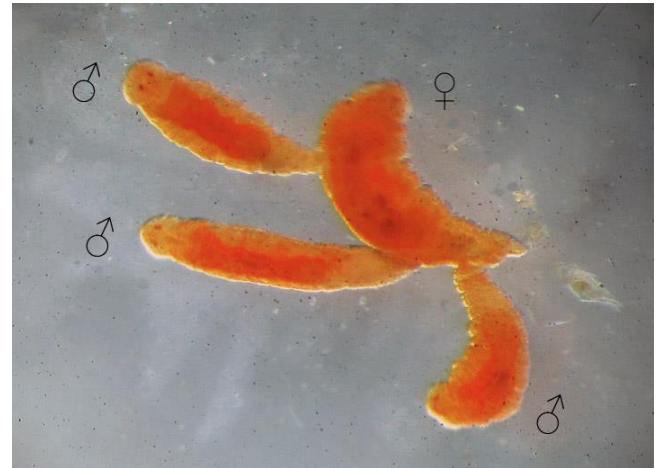


**ANNELIDA – *Dinophilus taeniatus***

*Dinophilus taeniatus* (Harmer, 1889) lives on filamentous algae inhabiting the lower tidal zone. They can be collected in the WSBS area at the Eremai rapids at low tide. It reproduces throughout the summer, when one or more males fertilize the female by piercing the wall of its body in the process of mating (Fig. 10). 1-2 days later the female lays the egg clutches. Individual eggs can be isolated using dissecting needles.

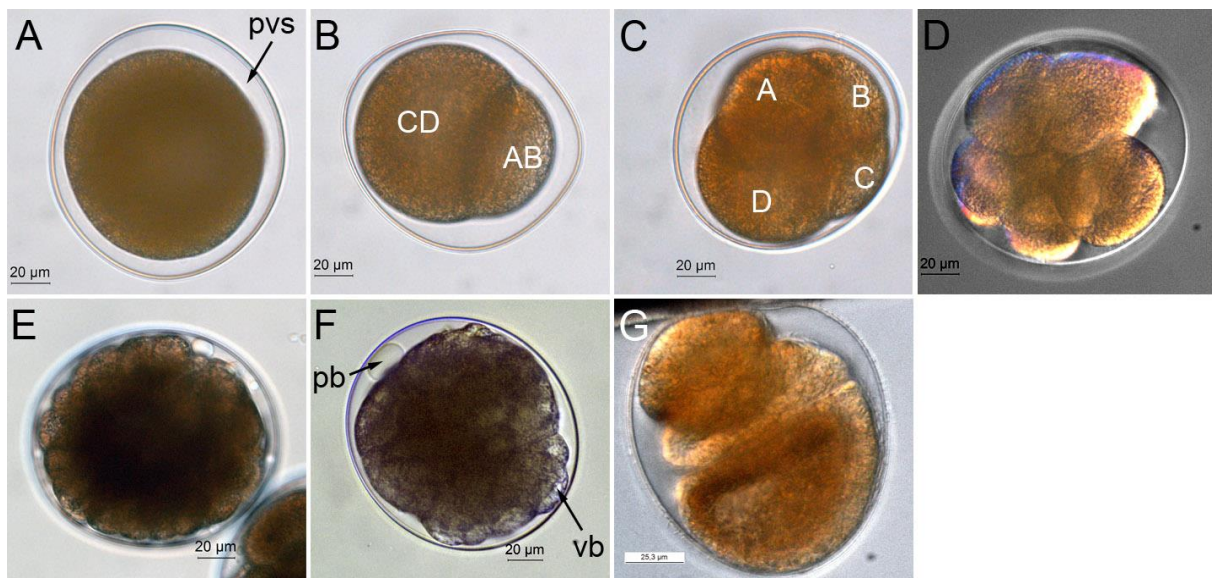
Cleavage of *D. taeniatus* is hetero-quadrant; thus, the first two blastomeres are extremely unequal and the blastomere CD greatly exceeds blastomere AB in size (Fig. 11 B). The division of AB is nearly equal, A being the slightly larger product, while the division of CD is highly unequal. All four cells formed after the second cleavage differs in size: D is prominent, whereas C, B and A are smaller and equal among one another (Fig. 11 C). At the four-cell stage of the *Dinophilus* ovum only one polar furrow is generally present, that at the animal pole, formed by the junction of A and C. This furrow is extremely long and turns to the right when viewed in the second cleavage plane. At the vegetal pole, all four cells meet at a point. Subsequent divisions occur in accordance with spiral-type cleavage.

During gastrulation large entodermal cells are immersed into the embryo (Fig. 11 E, F). Blastopore closes on the ventral side. The juvenile worm then forms in the egg membranes after some time (Fig. 11 G).



**Figure 10. Copulation of specimens of *Dinophilus taeniatus*.**

Three smaller males penetrates the single bigger female.

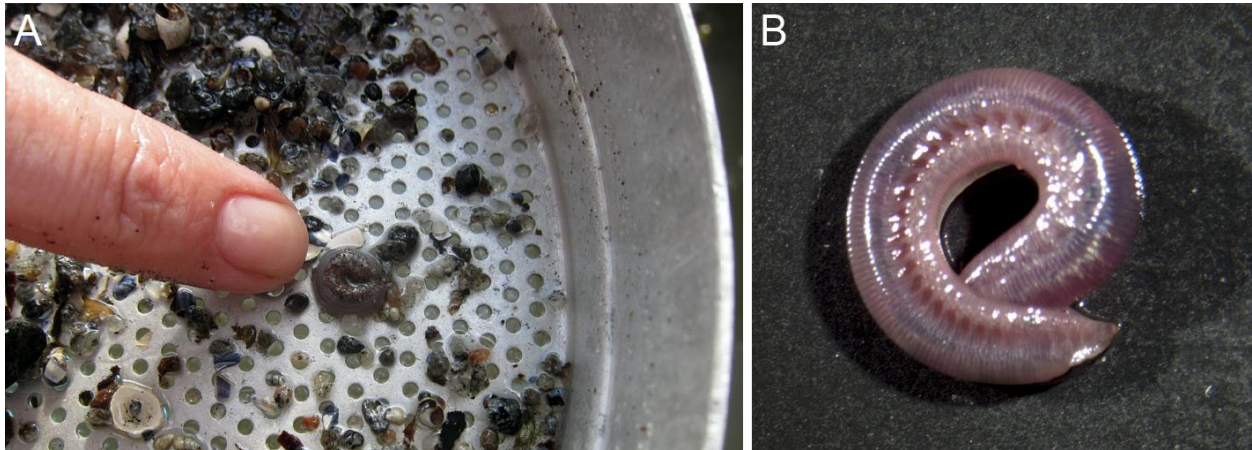


**Figure 11. Development of *Dinophilus taeniatus*.**

**A** – zygote, pvs – perivitelline space; **B** – 2-cell stage, blastomere CD is bigger than AB; **C** – 4-cell stage, blastomere D is bigger than the others; **D** – 8-cell stage; **E-F** – blastula stage, polar body (pb) and large vegetal (entodermal) blastomeres (vb) are visible; **G** – larva in the egg capsule.

**ANNELIDA – *Ophelia limacina***

Annelid *Ophelia limacina* (Rathke, 1843) inhabits the silty sands of tidal and upper subtidal zones. *Ophelia* burrows into the sediment. The worms can be extracted from the sediment by rinsing small particles of the sediment through a sieve (Fig. 12). The body cavity of adults is usually crammed with the gametes in June. Female gametes render the specimen yellowish, while male gametes effect a pinkish or purple colour. Gametes can be collected via puncturing of the body wall with a needle. A drop of sperm can be added to the egg suspension for *in vitro* fertilisation. The activation reaction and further embryonic development can be observed with a microscope.



**Figure 12. *Ophelia limacina*.**

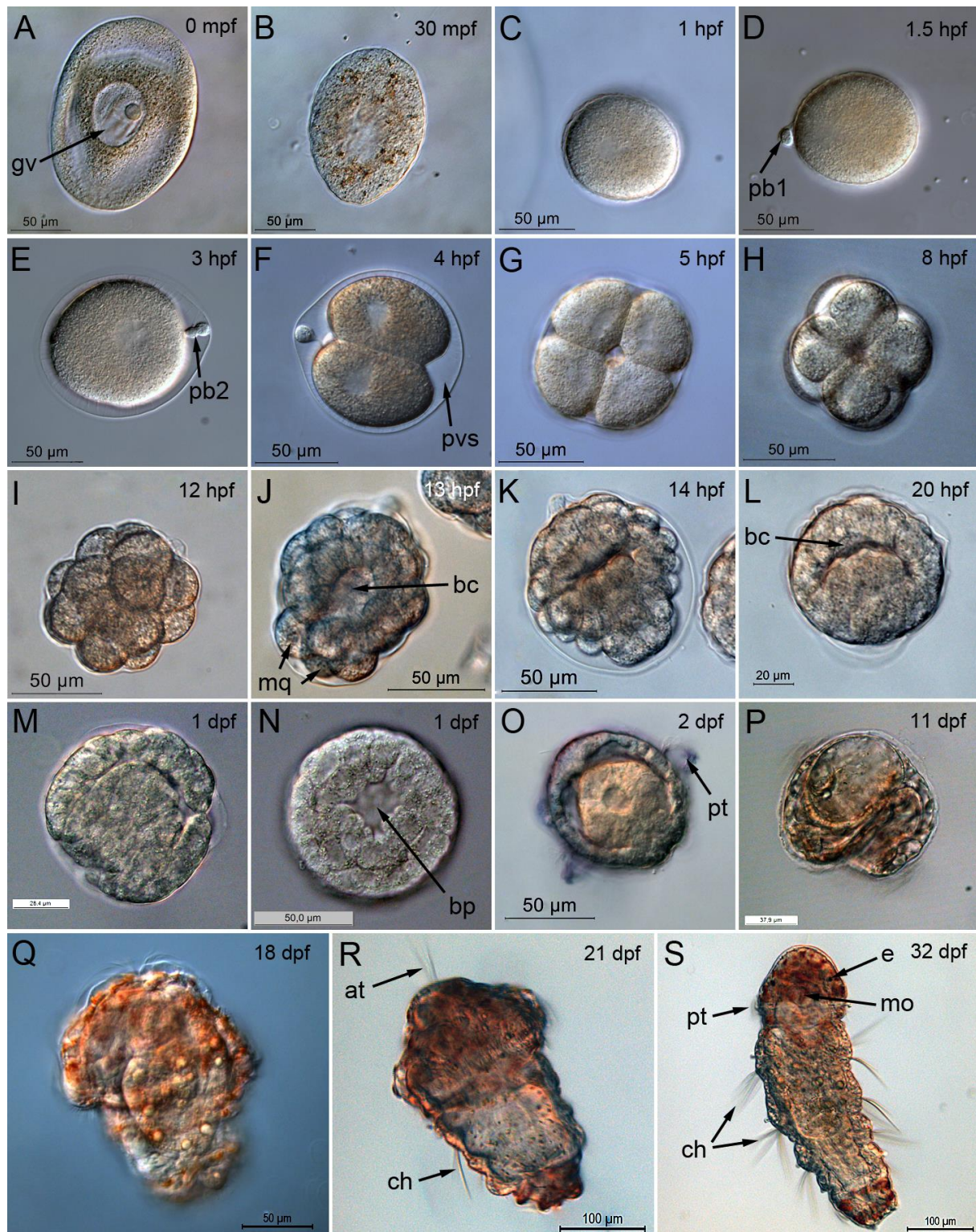
**A** – extraction the worms from the sediment with a sieve; **B** – rinsed adult worm.

Oocyte activation takes place within half an hour following fertilization. Germinal vesicle breakdown, cytoplasm reorganization (segregation) and the formation of a very thin fertilization envelope occur during this process (Fig 13 A, B). As a result, the initially flat, lentil-shaped egg gains a spherical shape (Fig. 13 C). Two polar bodies form within a couple of hours following fertilization (Fig 13 D, E) and cleavage starts (Fig 13 F).

*Ophelia* has classic homoquadrant spiral cleavage. The first two cleavage divisions are the meridional and divide the zygote into four equal blastomeres A-D (Fig. 13 G). As a result of the third cleavage, a quartet of vegetative macromeres (1A-1D) and a quartet of animal micromeres (1a-1d) appear (Fig 13 H). The animal blastomeres are shifted by 45 degrees in a clockwise direction, relative to vegetative blastomeres. This shift in the direction of blastomeres is characteristic of dextral spiral cleavage. Subsequent stages of spiral cleavage we will examine on molluscs.

The main quartet of blastomeres is visible as four large cells at the vegetal pole of the 64-cell embryo (Fig 13 J). These cells are immersed within the embryo (Fig. 13 K, L) and form the wide invagination (Fig. 13 M, N). Animal blastomeres continue dividing and begin moving over lateral sides of embryo and toward the vegetal pole. Gastrulation in *O. limacina* is a combination of invagination and epiboly. Later, the dorsal micromeres grow toward the ventral side and displace the blastopore in a ventro-anterior direction. Trochophora larva is formed shortly after the completion of gastrulation (Fig. 13 O). Stages of further larval development can be observed during the month following the fertilization (Fig. 13 P-S). The larvae should be fed by unicellular green algae.





**Figure 13. Development of *Ophelia limacina*.**

**A** – oocyte, germinal vesicle (gv) is visible; **B** – ooplasmic segregation; **C** – segregation finished; **D** – the first polar body (pb1); **E** – the second polar body (pb2); **F** – two-cell stage, perivitelline space (pvs) is visible; **G** – 4-cell stage, all the blastomeres are equal; **H** – 8-cell stage, spiral type is clearly distinguishable; **I** – 16-cell stage; **J** – 32-cell ceoloblastula, bc - blastocoel, the main quartet (mq) is on the vegetal pole; **K** – gastrulation starts, 4 vegetal blastomeres extend internally; **L** – mid-gastrula stage, bc – crescent blastocoel; **M** – late gastrula; **N** – late gastrula, vegetal surface view, blastopore (bp) is formed; **O** – trocho-phore, cilia of prototroch (pt) are visible; **P** – feeding trochophore; **Q** – early metatrochophore; **R** – metatrochophore, three segments, three pairs of chaetae (ch), apical tuft (at) are visible; **S** – settled juvenile, four segment worm, mo – mouth, e – eye, pt – prototroch, ch – chaetae.

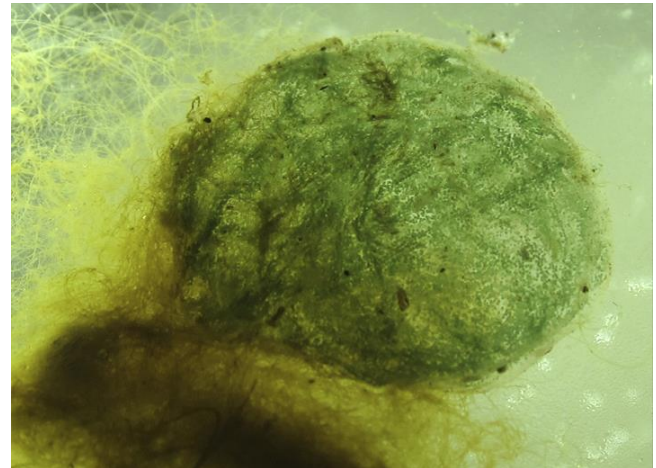


**ANNELIDA – *Phyllodoce maculata***

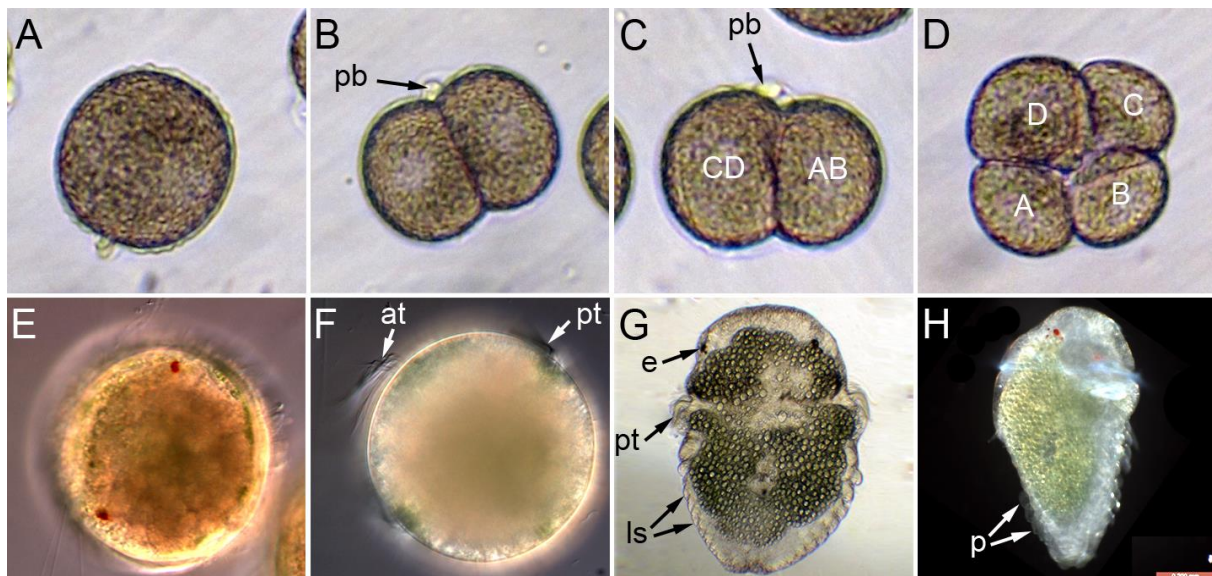
*Phyllodoce maculata* (Linnaeus, 1767) reproduces in late June. Females lay eggs in the form of green spherical mucous clutches attached to algae on the sandy tidal zone (Fig. 14). Individual eggs can be removed from the mucous capsules with a dissecting needle and cleavage can be observed with a microscope.

Like in all annelids, the eggs of *P. maculata* undergo spiral type cleavage (Fig. 15 A-D). D blastomere is generally larger than other blastomeres (Fig. 15 D), but it is often impossible to notice the difference. In general, after the first cleavage, CD blastomeres are bigger than AB (Fig. 15 C), but sometimes they are the same size (Fig. 15 B).

Trochophore larvae develop in the egg capsule (Fig 15 E, F). Trochophores have a prototroch and an apical organ (Fig 15 F). Two red eyes can be distinguished in an episphere (Fig 15 E). Larval segments and parapodia appear at later stages of free-floating metatrochophore and nectochaete (Fig. 15 G, H).



**Figure 14. The clutch of *Phyllodoce maculata*.**



**Figure 15. Development of *Phyllodoce maculata*.**

**A** – zygote; **B** – 2-cell stage, blastomeres AB and CD are equal, pb – polar bodies; **C** – 2-cell stage, blastomere CD is slightly bigger than AB, pb – polar bodies; **D** – 4-cell stage, blastomere D is bigger than the others; **E** – trochophore, view from the animal pole, red eyes are visible in an episphere; **F** – trochophore, lateral view, an apical tuft (at) and prototroch (pt) are visible; **G** – late trochophore, pt – prototroch, e – eye, ls – larval segments; **H** – nectochaete, p – parapodia.

**Figure 16. Trochophore larvae of White Sea polychaetes.** The figure is on the next page.

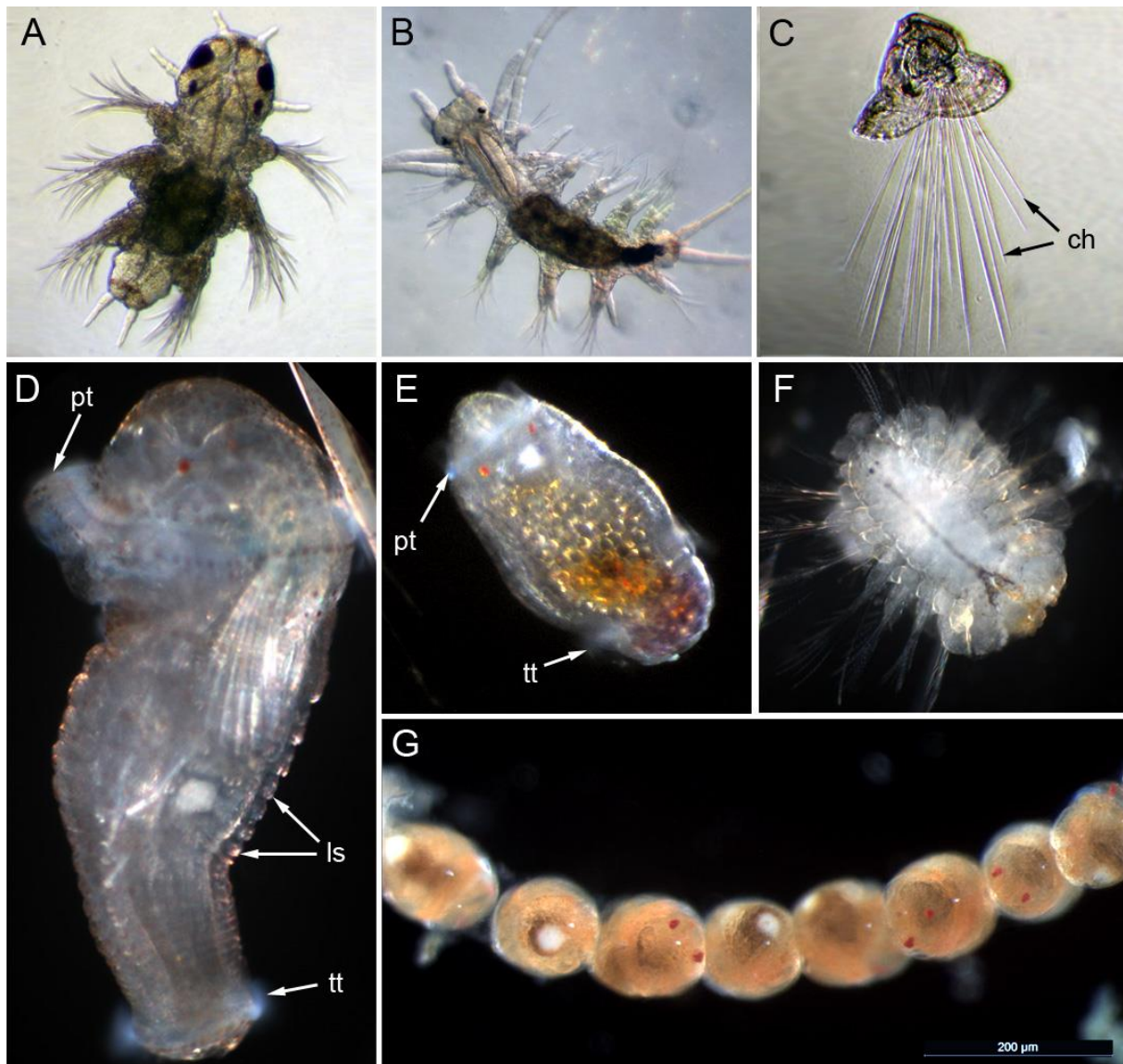
**A** – *Alitta (Nereis) virens* (Sars, 1835) nectochaeta with three larval segments; **B** – *Nereimyra (Castalia) punctata* (Muller, 1788) nectochaeta; **C** – *Galathowenia oculata* (Zachs, 1923) mitraria, ch – chaetae; **D** – *Pectinaria koreni* (Malmgren, 1866) nectochaeta, pt – prototroch, tt – telotroch, ls – larval segments; **E** – *Capitella capitata* (Fabricius, 1780) metatrochophore, pt – prototroch, tt – telotroch; **F** – *Harmothoe imbricata* (Linnaeus, 1767) nectochaeta, view from the ventral side, elytrae are visible behind the parapodia; **G** – chain of eggs of *Circeis armoricana* (Saint-Joseph, 1894) obtained from tube, trochophores with red eyes are inside eggs.

**ANNELIDA**

The vast majority of polychaetes have a planktonic larva stage during their life cycle. Details of larva structures vary among different species, but the overall plan of the body structure is the same.

Trochophore is the common stage of polychaetes development, which has some specific characteristics. It has a spherical or egg-shaped body that is subdivided into upper episphere and lower hyposphere by prototroch, a row of ciliated cells located along the equator. In addition to prototroch, larva can have a few more ciliary belts – anal telotroch and intermediate mesotrochs (Fig. 16 E). An apical sense organ (ciliary tuft) is located on the animal pole of larva. One or more pairs of eyes are located in the episphere. The mouth is located on the ventral side and often bordered by cilia. One more ciliary row, neurotroch, can be located along the ventral side of larva, below the mouth.

Metatrochophore is the next stage of the polychaete metamorphosis. The growth zone located in front of the anal lobe appears at this stage. Segmentation in the ectoderm of hyposphere and larval segments appear (Fig. 16 D). In the nectochaete stage, internal segmentation and typical biramous parapodia appear in the larvae (Fig. 16 A, B). Nectochaetes acquire head appendages, which are typical for the adult worms and sink to the seabed to begin benthic life.



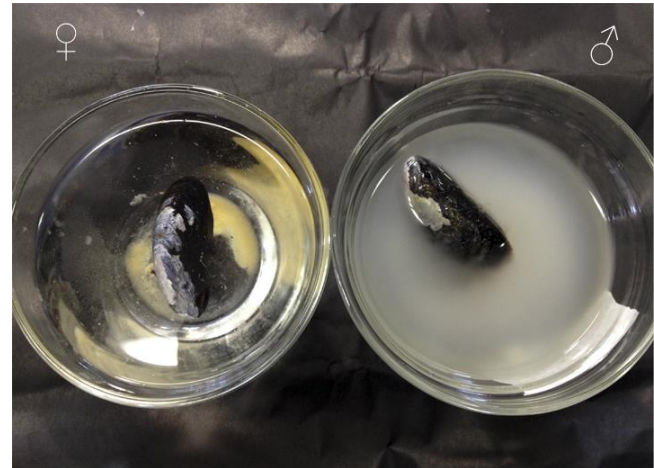
**Figure 16. Trochophore larvae of White Sea polychaetes.**

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**MOLLUSCA, BIVALVIA – *Mytilus edulis***

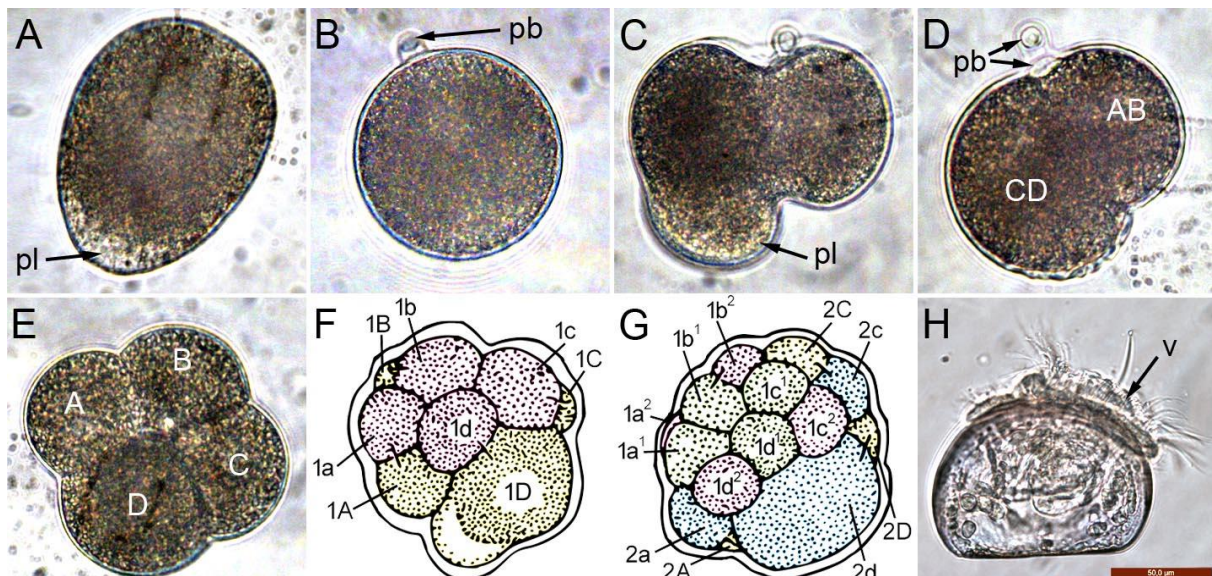
*Mytilus edulis* (Linnaeus, 1758) (mussels) is a widespread species and a convenient embryological object. These bivalves are dioecious and have external fertilization due to their sedentary lifestyle. The peak of their breeding season occurs at the beginning of July. Stimulation of mature individuals by a potassium chloride solution is used to obtain gametes. The injection of 0.5-2 ml of a 0.5 M KCl solution in the mantle cavity is carried out through the open shell valves. Stimulated molluscs should be placed in different containers to prevent polyspermy. Females spawn pinkish eggs and males release white sperm within an hour (Fig. 17). The eggs must be washed in several changes of filtered sea water and fertilized by adding a couple of drops of sperm.



**Figure 17. *Mytilus edulis* spawning after KCl injection.**

Unfertilized eggs have an irregular shape (Fig. 18 A), but soon become spherical after fertilization; a perivitelline space appears and polar bodies are formed (Fig. 18 B). Heteroquadrant cleavage with the formation of a polar lobe is typical for mussels. The polar lobe appears as a cytoplasmic protrusion at the vegetal pole prior to the first cleavage division (Fig. 18 C). Polar lobe material will become part of one of the blastomeres, which consequently will be larger (Fig. 18 D) and will further produce the coelomic mesoderm. The polar lobe is formed again prior to the second cleavage and enters blastomere D, which has the largest dimensions and is in contact with all three other blastomeres (Fig. 18 E). The polar lobe is formed once again prior to the third cleavage division.

During further development of the embryo, the formation of gastral invagination on the ventral side and the shell gland on the dorsal side occur. The larva has cilia at the animal and vegetal poles and several trochs. Shortly after that, the shell gland is everted, and veliger's bivalve shell is formed (Fig. 18 H).



**Figure 18. Development of *Mytilus edulis*.**

**A** – oocyte, the cytoplasm of polar lobe (pl) is visible at the vegetal pole; **B** – zygote, pb – polar body; **C** – the first cleavage division, pl – polar lobe; **D** – 2-cell embryo, blastomere CD is bigger than AB, polar bodies (pb) are visible; **E** – 4-cell embryo, blastomere D is bigger than the others; **F-G** – 8- and 16-cell embryos (Malachov, Medvedeva, 1985); **H** – veliger, v – velum.



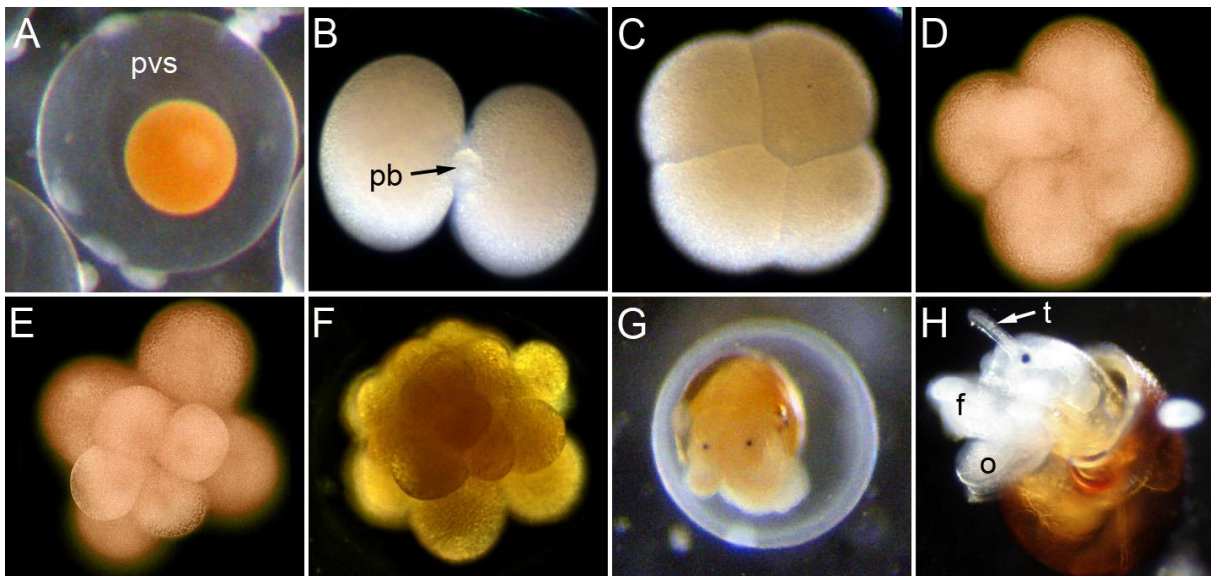
**MOLLUSCA, GASTROPODA – *Littorina saxatilis***

*Littorina saxatilis* (Olivi, 1792) is a small snail (up to 1 cm) that lives on stony and rocky grounds in the upper tidal zone (Fig. 19). These molluscs are dioecious and fertilization occurs internally. Fertilized eggs enter a special brood chamber of the female where their further development takes place. Embryonic material can be obtained by dissection of the mantle cavity using scissors and washing the former in Petri dishes with filtered seawater. During June-July, embryos at different stages of embryonic development can be found, from early cleavage to veliger.



**Figure 19. *Littorina saxatilis*.**

*L. saxatilis* cleavage is total (holoblastic) and equal (Fig. 20 B). The first two cleavage divisions are meridional and divide the zygote into four equal blastomeres A-D. One pair of opposite blastomeres, called B and D, touch onto the vegetal pole (Fig. 20 C), while the other pair (A and C) touches onto the animal pole. The typical spiral cleavage pattern is then initiated. The four daughter cells (micromeres) come to lie above each cleavage furrow of their mother cells at the animal pole. Each subsequent cleavage cycle results in a set of additional micromeres that involves a 45° twist of their mitotic spindle axes, relative to that of the mother cells (Fig. 20 D), but with alternating clockwise and counter-clockwise chirality between the generation of developing micromeres. As a result, the cells in the cleaving embryo appear spirally arranged when viewed from the animal pole (Fig. 20 E, F). The cross figure formed by two quartets of blastomeres is well visible at the animal pole of the 32-cell embryo. The blastopore arises in the form of a wide invagination at the vegetal pole and is later reduced to a narrow slit on the ventral side of the embryo. During this process, the macromeres are immersed deep into the invagination and dorsal side micromeres cover almost the entire surface of the embryo. Veliger larvae develop in the egg capsule (Fig. 20 G), but velum is weakly expressed and resorbed shortly before hatching of the juvenile snail (Fig. 20 H).



**Figure 20. Development of *Littorina saxatilis*.**

**A** – zygote, wide perivitelline space (pvs) is visible; **B** – 2-cell embryo, pb – polar body; **C** – 4-cell embryo, view from the vegetal pole, blastomeres D and B are in contact; **D** – the third cleavage division, view from the animal pole; **E** – 8-cell embryo, spiral pattern is clearly distinguishable; **F** – 16-cell embryo, quartets of blastomeres form tiers; **G** – incapsulated veliger; **H** – juvenile snail, t – tentacle, f – foot, o – operculum.

## MOLLUSCA

### GASTROPODA

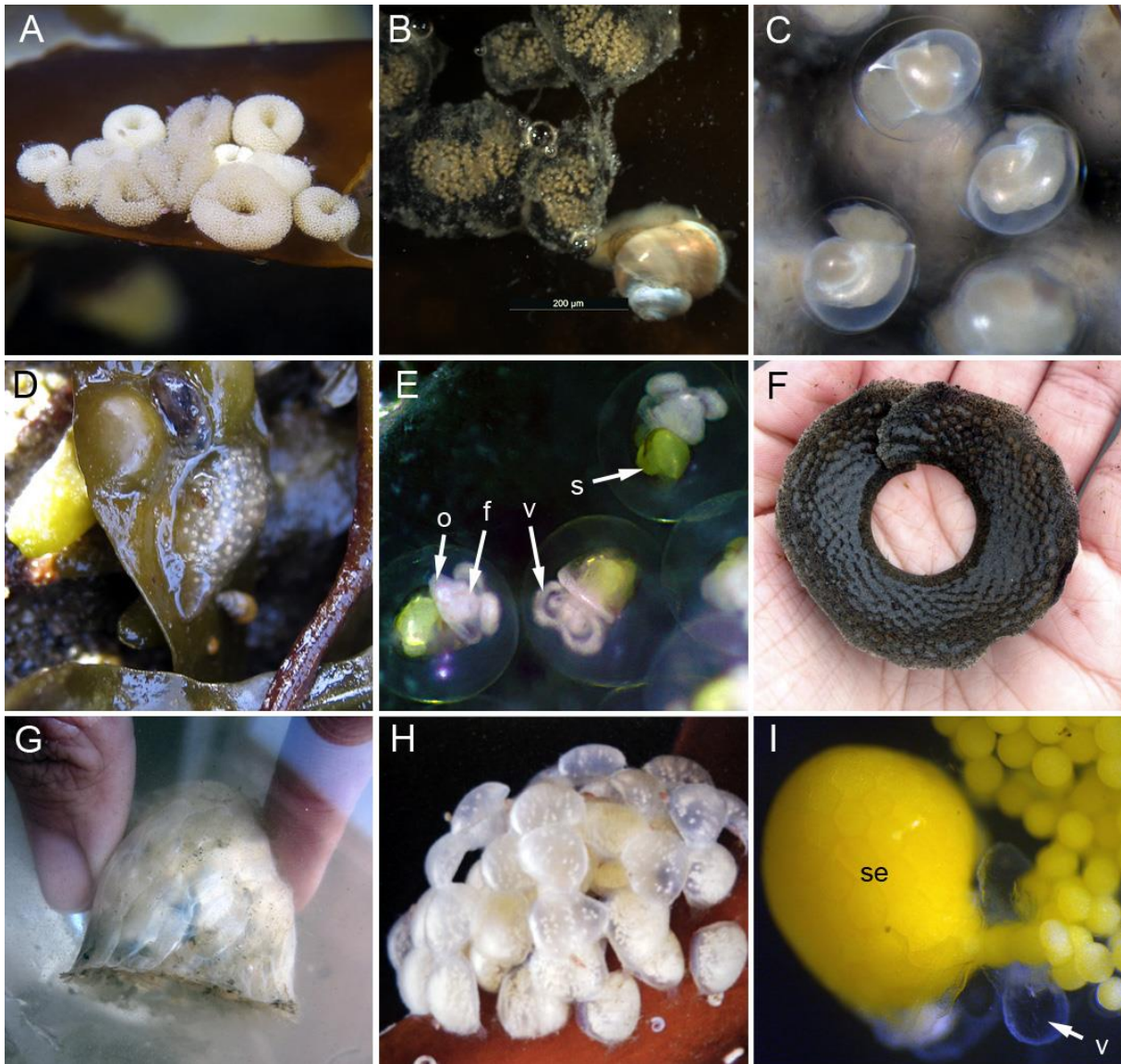
The majority of gastropods lay eggs in the form of a variety of clutches in June-July and can be used for spiral cleavage and veliger development observation. Clutches of several species are common on the thalluses of brown algae in the lower tidal and subtidal zones. Clutches of *Ephera vincta* (Montagu, 1803) have the form of an open ring and are common on laminaria (Fig. 21 A). *Margarites groenlandicus* (Gmelin, 1791) lays eggs in mucous clumps (Fig. 21 B). Clutches of *Littorina obtusata* (Linnaeus, 1758) are tough and have an oval shape (Fig. 21 D). Other gastropods lay their eggs on the seabed and among algae rhizoids. Clutches of *Cryptonatica affinis* (Gmelin, 1791) has the shape of tape twisted in the form of a truncated cone and are encrusted with sand (Fig. 21 F). *Neptunea despecta* (Linnaeus, 1758) and *Buccinum undatum* (Linnaeus, 1758) lay eggs in tight capsules, agglomerated in the form of a lump (Fig. 21 G, H). Hundreds of eggs are contained in each capsule but only a few embryos develop to veligers and are fed by the remaining eggs (Fig. 21 I). *Testudinalia tessulata* (Muller, 1776) is common on stones in the subtidal zone. Fertilization and development of larvae takes place in water. Spawning can be induced by turning the mollusc upside down and warming the water to room temperature (Fig. 22 E).

Nudibranchs lay typical clutches in the form of gelatinous strings containing egg capsules. Clutches of *Dendronotus frondosus* (Ascanius, 1774), *Coryphella verrucosa* (M. Sars, 1829), *Nudibranchus rupium* (Moller, 1842) and *Aeolidia papillosa* (Linnaeus, 1761) are common in June-July (Fig. 22 A-D).

From time to time, the veliger of sea angel *Clione limacina* (Phipps, 1774) can be found in plankton that have a pronounced velum and typical cup-shaped transparent shell (Fig. 22 H).

### LORICATA

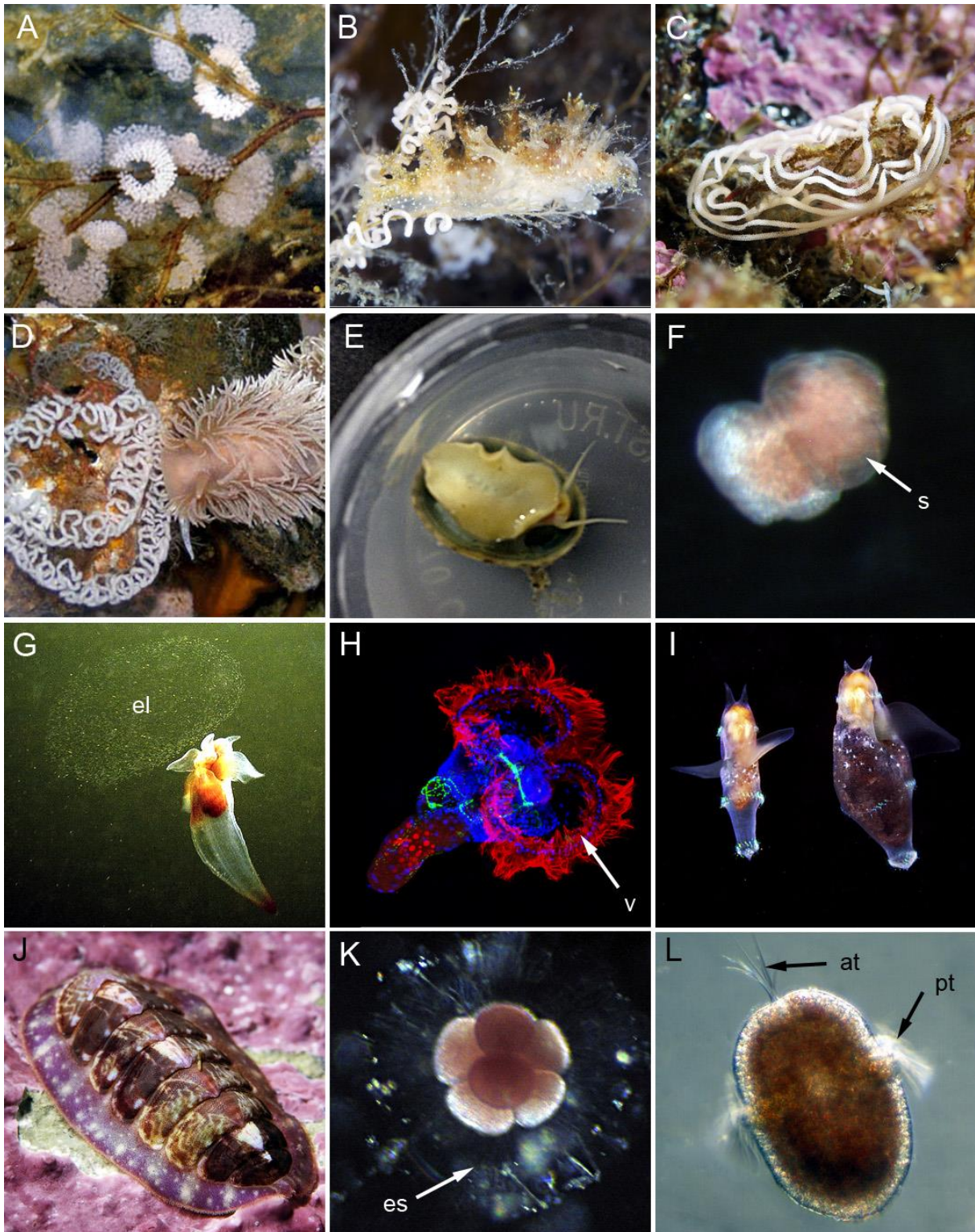
The chiton *Tonicella marmorea* (Fabricius, 1780) is widely distributed on rocky substrates in the subtidal zone (Fig. 22 J). Its eggs are coral-red in colour and are often found in plankton during late June to early July. They have a characteristic thick gelatinous shell that is penetrated by pores (Fig. 22 K). Eggs should be placed in a Petri dish with filtered seawater to observe typical spiral cleavage and further trochophore formation. Trochophores have a powerful prototroch and an apical tuft (Fig. 22 L), and remains in the egg shells for a long time before hatching. When settling, the foot appears on the ventral side and the germ of the segmented sink appears on the dorsal side. The prototroch and the apical tuft are reduced during metamorphosis.



**Figure 21. Development of White Sea molluscs.**

**A** – *Epheria vincta* egg clutches; **B** – *Margarites groenlandicus* with egg clutches; **C** – incapsulated veligers of *M. groenlandicus* with transparent shells; **D** – *Littorina obtusata* egg clutch; **E** – incapsulated veligers of *L. obtusata*, v – velum, f – foot, o – operculum, s – shell; **F** – *Cryptonatica affinis* egg clutch; **G** – *Neptunea despecta* egg clutch; **H** – *Buccinum undatum* egg clutch; **I** – *B. undatum* veliger eats the undeveloping eggs in egg capsule, swallowed eggs (se) are visible in veliger's stomach, v – velum.





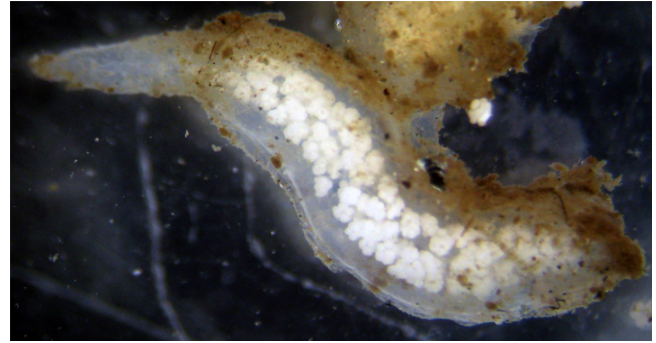
**Figure 22. Development of White Sea molluscs.**

**A** – *Nudibranchus rupium* egg clutches on the hydroid colonies; **B** – *Dendronotus frondosus* with its egg clutch; **C** – egg clutch of *Coryphella verrucosa*; **D** – *Aeolidia papillosa* with its egg clutch; **E** – *Testudinalia tessulata* inverted male releases the sperm; **F** – *T. tessulata* veliger larva, s – shell; **G** – *Clione limacina* with its pelagic egg clutch (el); **H** – *C. limacina* trochophore fluorescent micrograph (nuclei are blue, cilia are red, serotonin neurons are green), v - velum; **I** – *C. limacina* polytrochal larvae; **J** – adult *Tonicella marmorea*; **K** – *T. marmorea* cleavage embryo in egg shell (es); **L** – trochophore, at – apical tuft, pt - prototroch.



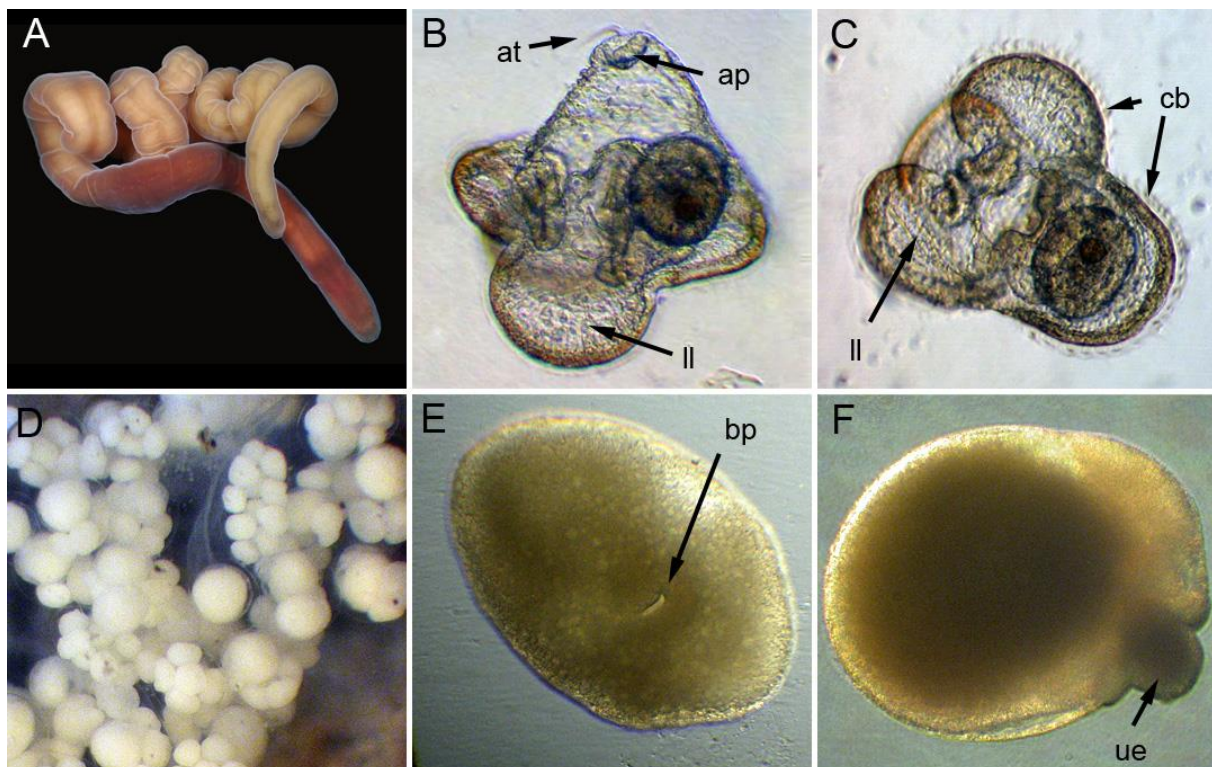
**NEMERTEA**

Several species of nemerteans abound under rocks and seaweeds on silty substrates in the tidal zone. *Ramphogordius sanguineus* (Rathke, 1799) (Fig. 24 A) reproduces mainly asexually, by fragmentation. They are dioecious and fertilization is external. Planctonic pilidium larvae are formed then (Fig. 24 B, C). Pilidium larvae have a helmet shape with two lateral lobes and an apical plate with a cilia tuft (Fig. 24 B). The ciliated band, which is homologous to prototroch, is located at the edge of the lower surface and the lateral lobes of the larva (Fig. 24 C). The adult worm body is formed from the imaginal discs during a catastrophic metamorphosis.



**Figure 23. *Poseidon ruber* egg clutch.**

Another widespread nemertean, *Poseidon ruber* (Muller, 1774), produces elongated mucous clutches containing 100-250 egg capsules (Fig. 23). Each egg capsule contains about 10 eggs, but only 1-2 of these develop (Fig. 24 D). The embryo forms a mouth (Fig. 24 E) that swallows all other eggs (Fig. 24 F). The fully formed worm leaves the egg shell then.

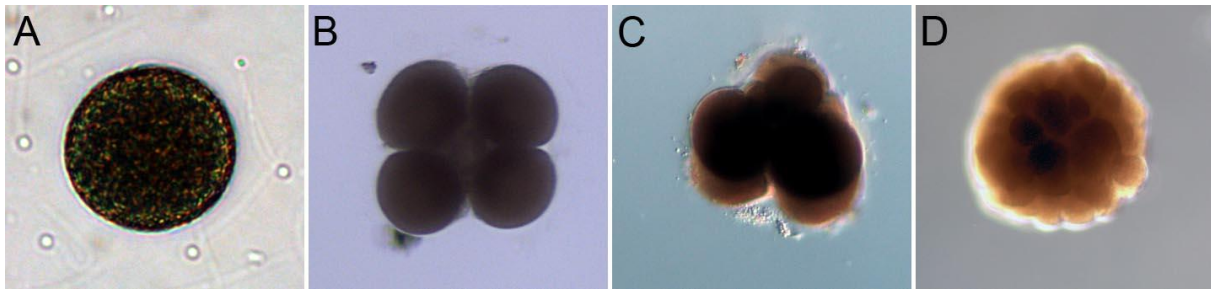


**Figure 24. Development of White Sea nemerteans.**

**A** – *Ramphogordius sanguineus* adult (from [www.aphotomarine.com](http://www.aphotomarine.com)); **B** – *R. sanguineus* pilidium larva, lateral view, ap – apical plate, at – apical tuft, ll – lateral lobe; **C** – *R. sanguineus* pilidium larva, lateral from lower surface, ll – lateral lobe, cb – ciliated band; **D** – *Poseidon ruber* egg capsules in the egg clutch, developing embryos are bigger than undeveloping ones; **E** – *P. ruber* embryo, bp – blastopore; **F** – *P. ruber* embryo swallows the undeveloping egg (ue) through the blastopore.

**BRACHIOPODA**

*Hemithiris psittacea* (Gmelin, 1792) is the only species of brachiopods in the White Sea. Its full development has to date not been described. It is known that holoblastic radial cleavage and coeloblastula formation are characteristic for its development (Fig. 25). Details of gastrulation and larval development remain unknown.



**Figure 25. Development of *Hemithiris psittacea*.**

**A** – zygote, sperms are visible around; **B** – 4-cell embryo; **C** – the third cleavage division, blastomeres are unequal; **D** – morula-early blastula.

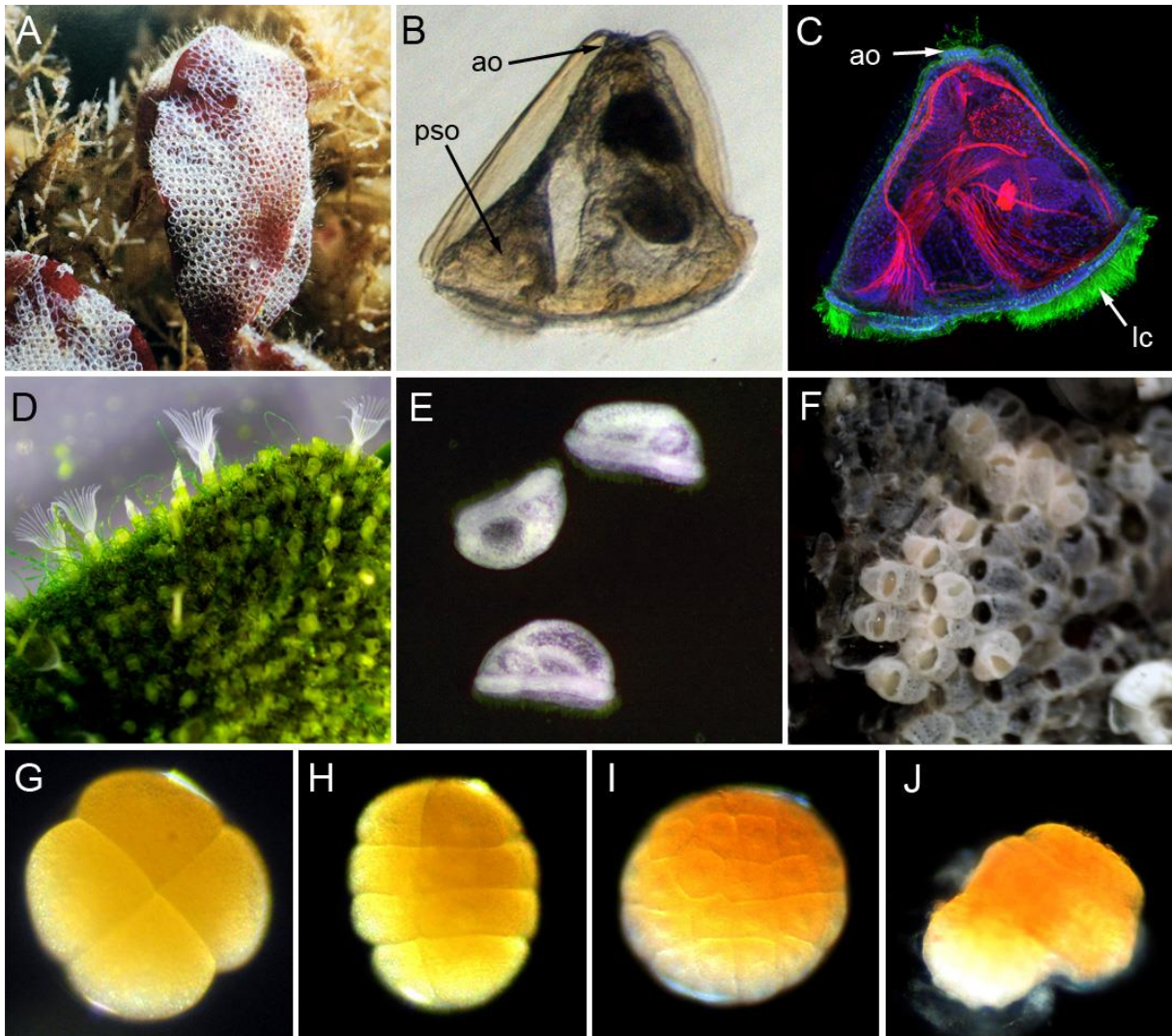
**BRYOZOA**

Colonies of *Cribrilina annulata* (Fabricius, 1780) are often presented on the red algae thalluses in the subtidal zone (Fig. 26 F). Developing embryos can be found in the ovicells throughout summer. One should remove individual embryos from ovicells with dissecting needles for observing embryonic development. Cleavage is complete (holoblastic) and almost uniform (Fig. 26 G). The first three divisions occur in perpendicular planes and divide the zygote into eight equal blastomeres. The following two divisions occur in parallel to the first two; the result is a two-layer plate, wherein the blastomeres are arranged in rows (Fig. 26 H, I). Later, the embryo acquires the form of a biconvex lens with a cavity (placula stage). Four vegetative cells are immersed into the embryo during gastrulation and form the entoderm and mesoderm. Lecithotrophic (non-feeding) coronate larvae (Fig. 26 J) covered with cilia develop then. The larvae have a sensory region called the apical organ on its anterior end. The internal sac is visible through the body wall at the broader posterior end of the larva. This invagination is everted during metamorphosis, helps the larva attach to the substratum and makes up a significant portion of the epidermis of the founding zooid of the colony.

*Electra pilosa* (Linnaeus, 1767) is common on algae thalluses in the subtidal zone (Fig. 26 A). Planktotrophic cyphonaute larvae occur in plankton throughout the summer. Cyphonaute body has the shape of a flattened cone and is enclosed in a bivalve shell (Fig. 26 B). The apical organ with its sensitive cilia is located at the top of the cone. Powerful locomotor cilia are located on the edge of the base of the cone (Fig. 26 C). The larva has an atrium where the mouth and anus open. The pear-shaped organ is situated at the front part of the larval base. It has sensitive cilia, is connected to the apical organ and performs a sensory role in the selection of the substrate during metamorphosis. The internal sac is located in front of the anus and releases a sticky secretion, required for attaching the larva during metamorphosis.

*Frustrellidra hispida* (Fabricius, 1780) form colonies in the form of sleeves on brown algae (Fig. 26 D). The larvae are similar to cyphonaute (Fig. 26 E), but have an elongated shape, reduced gut and swim a very short time.





**Figure 26. Development of White Sea bryozoans.**

**A** – *Electra pilosa* colony; **B** – *E. pilosa* cyphonaute larva, ao – apical organ, pso – pear-shaped organ; **C** – fluorescent micrograph of *E. pilosa* cyphonaute (nuclei are blue, cilia are green, muscles are red), ao – apical organ, lc – locomotor cilia; **D** – *Frustrellidra hispida* colony; **E** – *F. hispida* larvae; **F** – *Cribrilina annulata* colony with embryos in the ovicells; **G-J** – embryonic development of *C. annulata*; **G** – 4-cell embryo; **H, I** – cleavage; **J** – coronate larva.

### NEMATODA – *Pontonema vulgare*

*Pontonema vulgare* (Bastian, 1865) is a large (12-20 mm in length) nematode that inhabits the plexuses of rhizoids in the subtidal zone, as well as filamentous algae and silt under rocks in the lower tidal zone. Nematodes mate in early summer, but fertilized egg development is stopped for a few months. The eggs are clearly visible through the transparent cuticle of females (Fig. 27). Eggs can be sampled via dissection of the female using two dissecting needles. The egg is activated and the fertilization envelope is formed within roughly half an hour (Fig. 28 B). A newly formed fertilization envelope is sticky and adheres to the substrate. It is convenient to put the eggs on the cover slip placed on the bottom of the Petri dish with sea water.



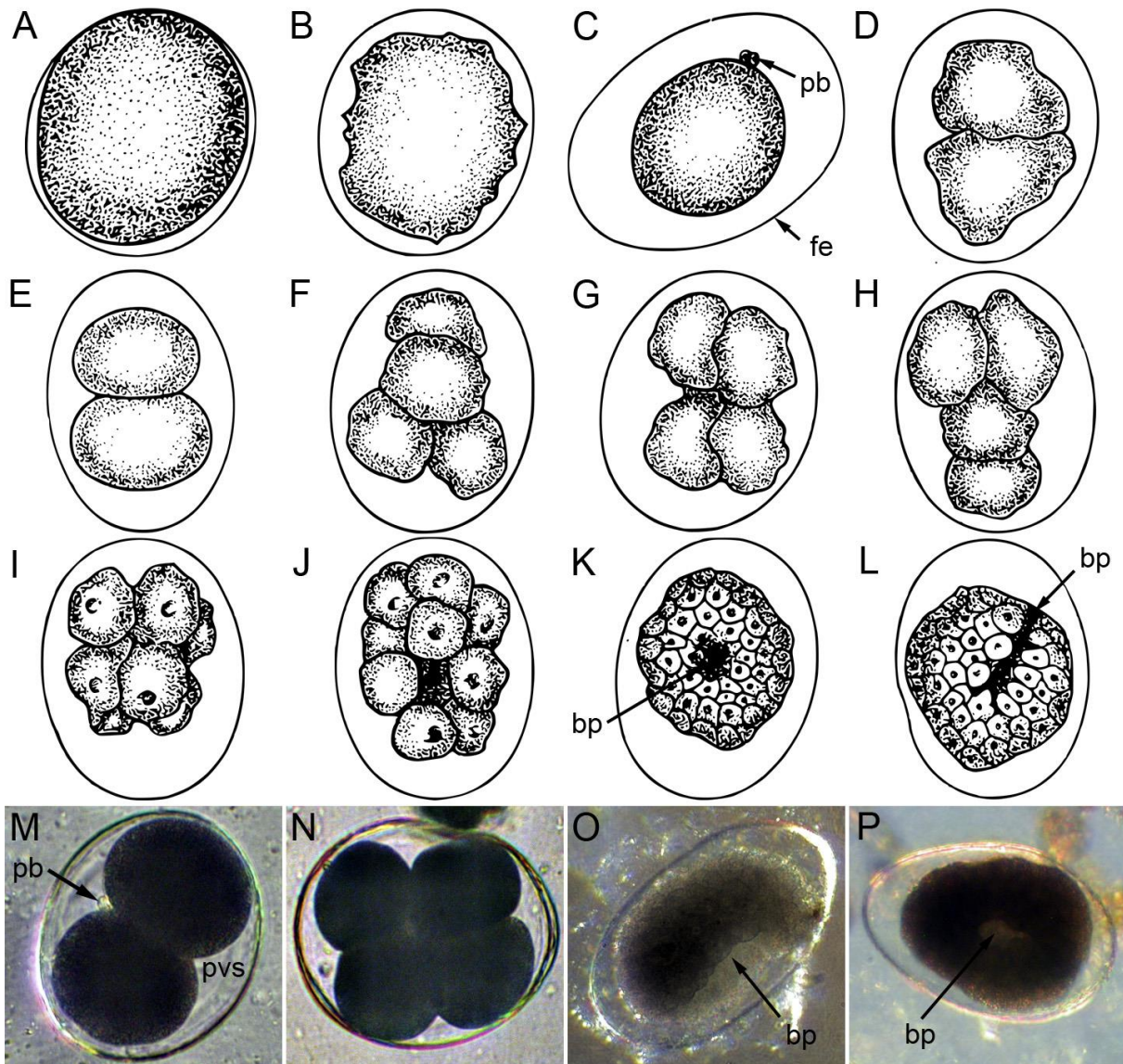
**Figure 27. *Pontonema vulgare* female.**

Eggs (e) are visible inside the worm.

Polar bodies appear in about three hours after activation (Fig. 28 C). The first cleavage occurs 20 hours after activation (Fig. 28 D). Blastomeres have a constantly changing irregular shape in the intervals between divisions. However, they take the form of a ball immediately prior to division (Fig. 28 E, M). Blastomeres have different configurations after the second cleavage (Fig. 28 F-H), but finally take on a rhombus pattern (Fig. 28 N). Cleavage becomes asynchronous after the 16-cell stage. One of the blastomeres lags during divisions, deepens and forms the endoderm (Fig. 28 J). The remaining blastomeres cover it and form a blastopore (Fig. 28 K, O). Later, the blastopore takes a slit-like shape (Fig. 28. L, P) and splits into oral and anal openings.

In the White Sea Biological Station, nematode development can also be observed among two other species – *Metachromadora vivipara* (Filipjev, 1918) and *Enoplus brevis* (Bastian, 1865).





**Figure 28. Development of *Pontonema vulgare*.**

**A-L** – embryonic development (Belousov, Dabagyan, 1992); **A** – oocyte; **B** – cortical reaction; **C** – zygote, fertilization envelope (fe) and two polar bodies (pb) are visible; **D** – 2-cell embryo; **E** – 2-cell embryo immediately before the second cleavage division; **F-H** – 4-cell embryos that form tetrahedron, rhombus and T-shape patterns; **I** – 8-cell embryo; **J** – 16-cell embryo; **K** – gastrula with wide blastopore (bp); **L** – gastrula with slit-shaped blastopore (bp); **M** – 2-cell embryo, pvs – perivitelline space, pb – polar body; **N** – 4-cell embryo; **O** – gastrula with wide blastopore (bp); **P** – gastrula with slit-shaped blastopore (bp).

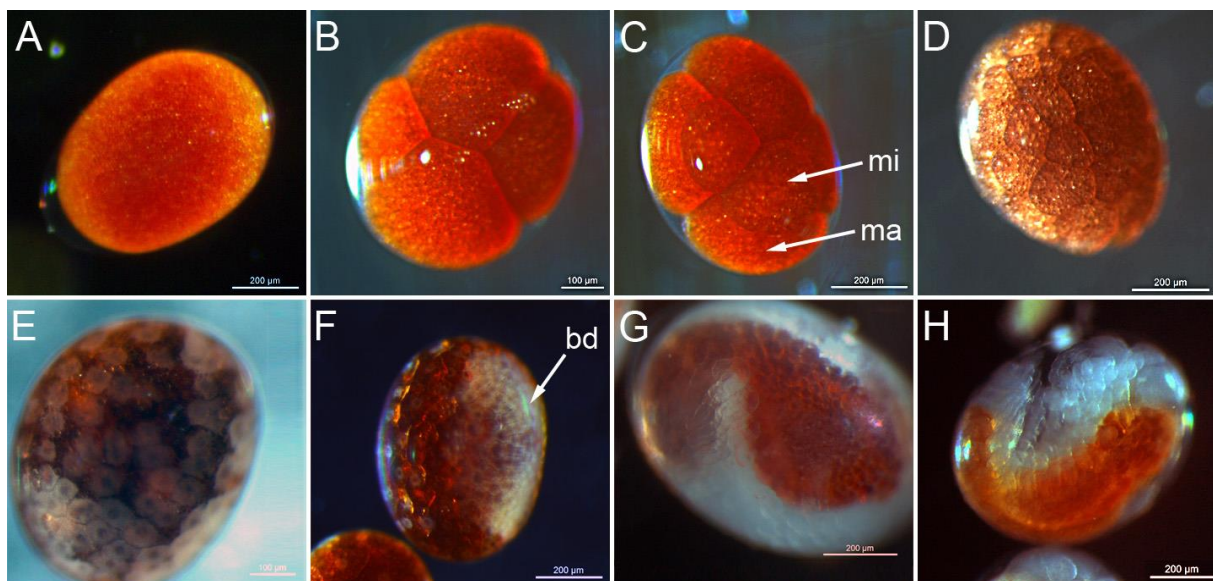
**ARTHROPODA, CRUSTACEA – *Marinogammarus obtusatus***

Two species of amphipods are most common around the WSBS. *Gammarus duebeni* (Lilljeborg, 1851) inhabits the tidal zone, while *Marinogammarus obtusatus* (Dahl, 1938) is typically found in the lower tidal, in bush fucus. The structure of the uropod is the most reliable sign for distinguishing between the two species. Amphipods copulate and are held together for a long time (Fig. 29). The female lays eggs immediately after the moult and the male fertilizes them. Egg development occurs in the female's brood chamber for a period of three weeks. Mating amphipods should be kept in a Petri dish at a constant temperature and the water changed every 2-3 days. The male should be removed as soon as possible. Eggs selected for observation can be removed from the brood chamber using dissecting needles; however, the development of the extracted eggs lasts only up to the stage of early gastrula.



**Figure 29. Mating of *Marinogammarus obtusatus*.**

The eggs of amphipods contain significant amounts of yolk (polylecithal) that is orange-red (Fig. 30 A) or gray-green in colour. Formation of polar bodies and perivitelline space occurs within one hour after moulting of the female. First, a complete (holoblastic) cleavage occurs. The first and second cleavage furrows occur about four and seven hours after fertilization, and divide the zygote into four approximately equal blastomeres (Fig. 30 B). The third division furrow appears roughly nine hours after fertilization and forms four macromeres and four micromeres, the relative position of which can be both spiral and radial. During the following cleavage, blastomeres' dimensions equalize and morula is formed (Fig. 30 D). Blastoderm formation begins about 36 hours after fertilization. Nuclei, surrounded by whitish cytoplasm, emerge on the blastomeres' surface (Fig. 30 E) and are subsequently separated from the yolk mass. These surface cells form the blastoderm (Fig. 30 F), continue to divide and gradually surround the yolk mass. Germinal disc formation, gastrulation and morphogenesis occur in the later stages of development (Fig. 30 G, H).



**Figure 30. Development of *Marinogammarus obtusatus*.**

**A** – zygote; **B** – 4-cell stage; **C** – 8-cell stage, macromeres (ma) and micromeres (mi) are formed; **D** – morula; **E-F** – blastoderm (bd) formation; **G-H** – segmented late embryos.



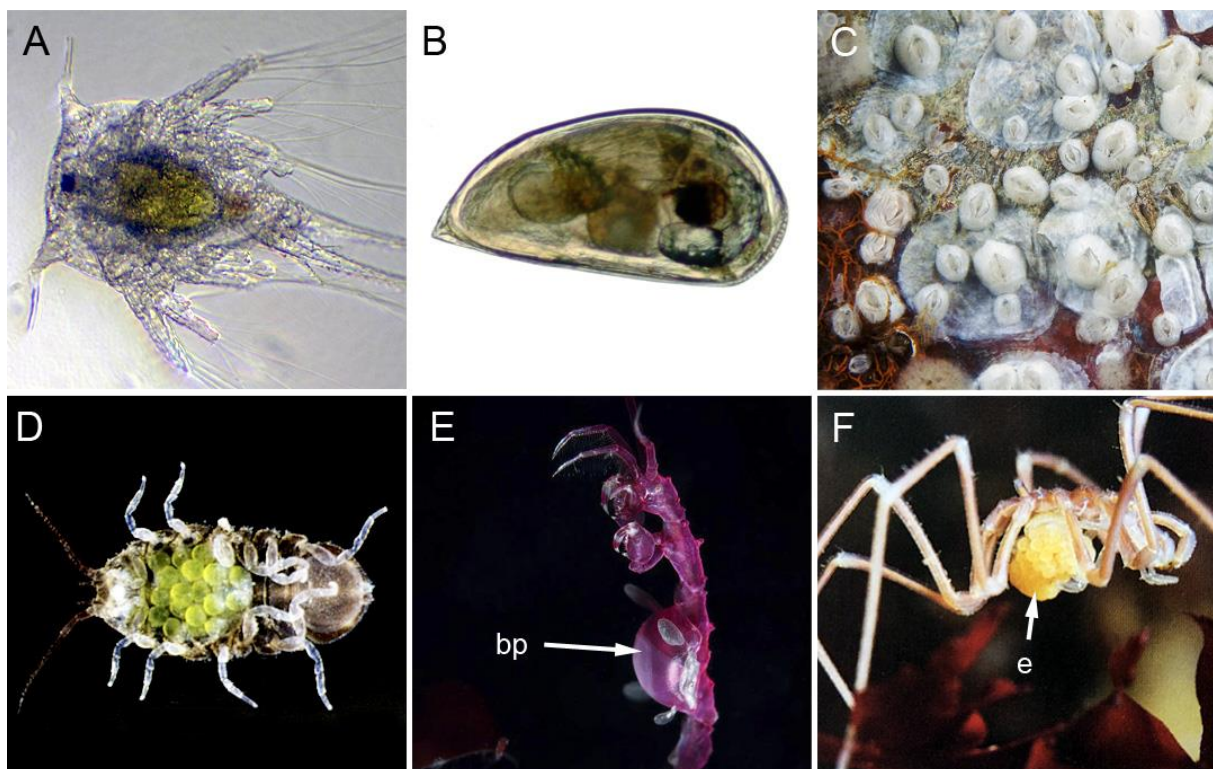
**ARTHROPODA****CRUSTACEA**

The fauna of the White Sea is rich in crustaceans, including planktonic ones from the orders Cladocera, Cyclopoida and Calanoida. Their adult and larval stages are abundant in plankton samples. Nauplius is the earliest larval stage, where the body consists of the head division only and has one eye and three pairs of appendages: non-branched antennules, biramous antennae and mandibles. After moulting, the segmented thoracic division with its corresponding appendages appears and the larva is called metanauplius. Abdominal segments appear in the later stages of development. Nauplii of barnacles from order Cirripedia – *Semibalanus balanoides* (Linnaeus, 1758), *Balanus balanus* (Linnaeus, 1758) and *Verruca stroemia* (O.F. Muller, 1776) – are found in large numbers in plankton at the beginning of the summer (Fig. 31 A). They have a typical dorsal shield with tail needle and lateral horns. Following the metanauplius stage, a cyprid larva (Fig. 31 B) is formed that does not feed and which soon settles on a rocky substrate using antennules, and metamorphoses (Fig. 31 C).

Development of isopods can be observed in *Jaera albifrons* (Leach, 1814). These isopods are often found in desalinated areas and produce green eggs in the brood pouch (Fig. 31 D), where they develop. Sea goats – *Caprella septentrionalis* (Kroyer, 1838) – are amphipods that live on red algae. The development of eggs takes place directly in the brood pouch (Fig. 31 E).

**PYCNOGONIDA**

Sea spiders – *Nymphon longitarse* (Kroyer, 1845), *Nymphon grossipes* (Fabricius, 1780) and *Phoxichilidium femoratum* (Rathke, 1799) – reproduce in the middle of summer. In July, most of the males carry the eggs on their ovigerous legs (Fig. 31 F). Eggs are polyecital and development is direct. Protonymphon larvae with three pairs of appendages hatch and acquire the rest of their segments consequentially during further development.



**Figure 31. Development of White Sea arthropods.**

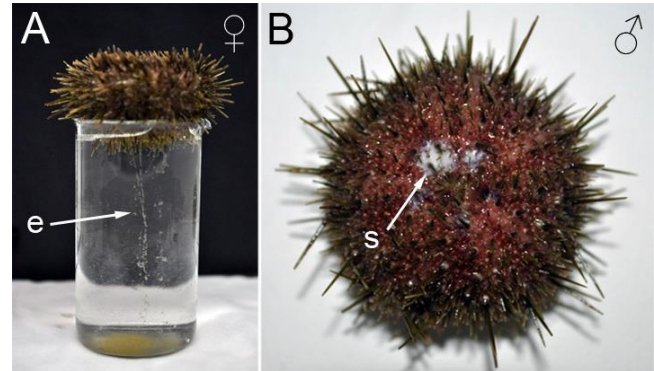
**A-C** – development of cirripedians; **A** – nauplius; **B** – cyprid larva (M. C. Mingorance Rodríguez via Wikimedia Commons); **C** – settled juvenile barnacles; **D** – *Jaera albifrons* with green eggs; **E** – *Caprella septentrionalis*, bp – brood pouch; **F** – *Nymphon grossipes* male with the eggs (e) on the ovigerous legs.



**ECHINODERMATA, ECHINOIDEA – *Strongylocentrotus pallidus***

*Strongylocentrotus pallidus* (G.O. Sars, 1871) is the only species of sea urchins in the White Sea. The only possibility for obtaining them is through a diving service. Sea urchins are dioecious, their reproduction through external fertilization occurring in the middle of summer. Since they live in deep-sea habitats, adult sea urchins and their embryos cannot endure warmth and must be kept in a cold temperature of no more than 8°C. To induce spawning, inject 0.5 M KCl into the body cavity through the perioral membrane. Gametes will start being released from gonopores on the aboral side within 5-10 minutes. Put the female, which releases orange eggs, on a glass with filtered seawater, aboral side down, so that the eggs can immediately enter the water (Fig. 32 A). Spawning

usually lasts for 20-30 minutes. Obtained eggs should be washed with 2-3 changes of filtered seawater. The male releases white sperm (Fig. 32 B), which can be used for fertilization. If sperm is to be stored, dissect the male shell using scissors, cut the testes and store it in a refrigerator in a sealed tube. In vitro fertilization is performed by adding a drop of sperm to the egg suspension.

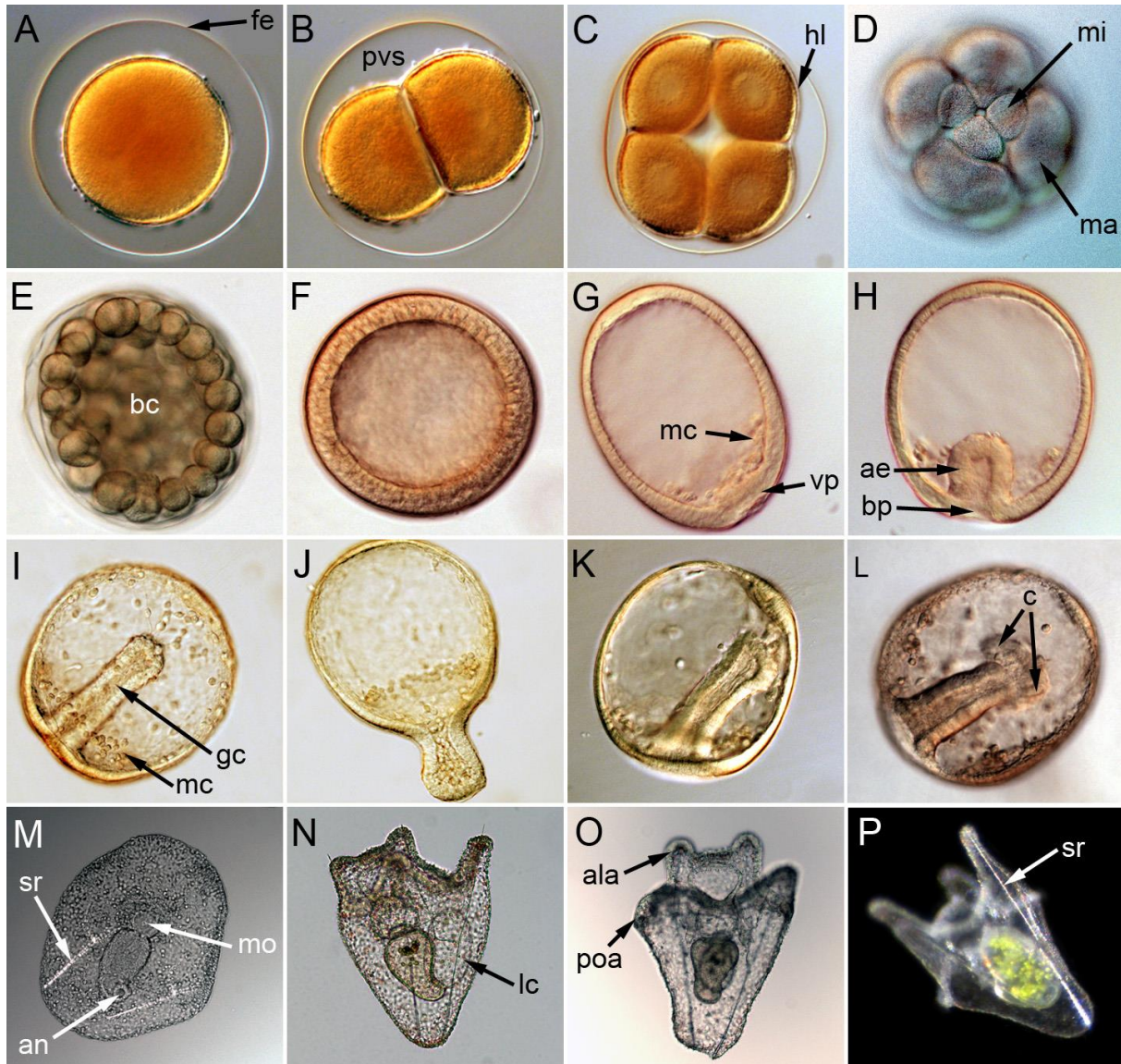


**Figure 32. Obtaining of the gametes of sea urchin *Strongylocentrotus pallidus* by KCl injection.**

**A** – spawning female, **e** – eggs; **B** – spawning male, **s** – sperm.

A fertilization envelope occurs within 5-10 minutes following fertilization (Fig. 33 A). Cleavage is holoblastic and radial (Fig. 33 B, C); thus, the first three cleavage furrows divide the embryo into eight equal blastomeres. Unevenness appears at the fourth cleavage; thus, the animal blastomeres are divided meridionally into eight mesomers and vegetal blastomeres are divided into four macromeres and four micromeres lying on the vegetal pole (Fig. 33 D). The unevenness of blastomeres disappears by the blastula stage (Fig. 33 E). Due to the low temperature at which they live, the development of sea urchins is very slow. The first and second cleavage occurs after 10 and 15 hours after fertilization, epithelial blastula is formed after four days (Fig. 33 F) and hatching of mesenchyme blastula occurs after seven days (Fig. 33 G). In the process of gastrulation, immigration of mesenchymal cells and the formation of the archenteron by invagination occurs (Fig. G-I). The archenteron grows in the direction of the vegetal side of the future larva (Fig. 33 K) and coelomic pouches are formed later by separating from the top of archenteron (Fig. 33 L).

A mouth breaks through on the ventral side of the larva and skeletal spicules are formed from mesenchymal cells in the prism stage (Fig. 33 M). Echinopluteus larvae are formed at a later stage (Fig. 33 N-P). Each of the four arms of the early pluteus has a skeletal spicule inside (Fig. 33 P) and is covered with a ciliated band. Rudiments of the juvenile sea urchin appear as a result of the differentiation of coeloms and metamorphosis occurs later.



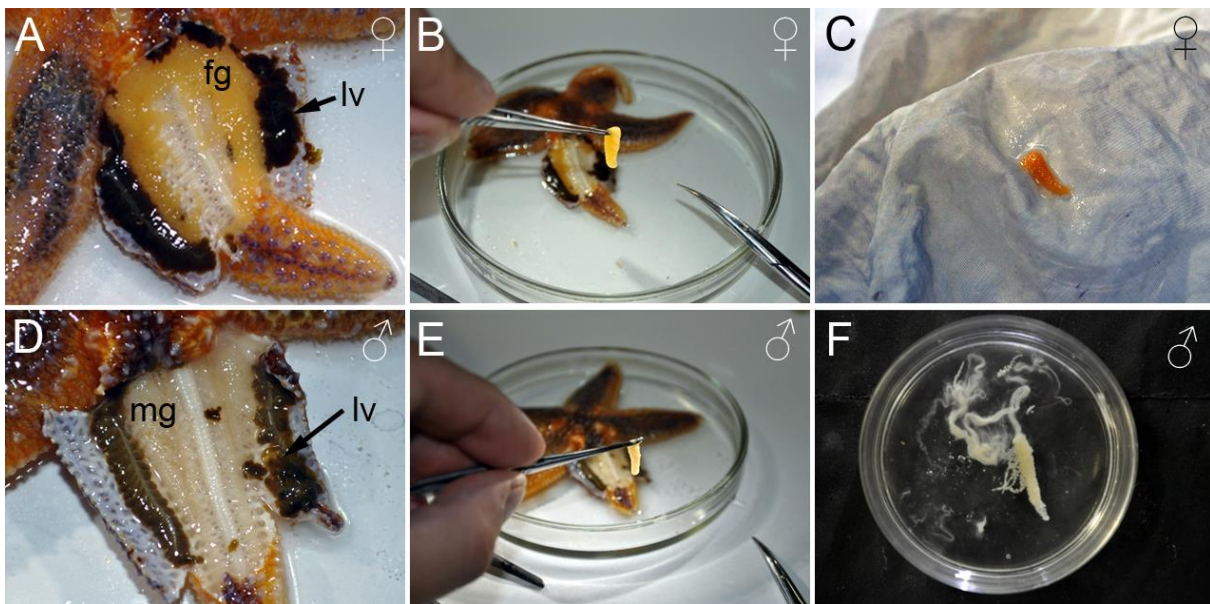
**Figure 33. Development of *Strongylocentrotus pallidus*.**

**A** – zygote, fertilization envelop (fe) is visible; **B** – 2-cell stage, pvs – perivitelline space; **C** – 4-cell stage, hyaline layer (hl) is visible; **D** – 16-cell embryo, view from vegetal pole, mi – micromeres, ma – macromeres; **E** – early blastula, bc – blastocoel; **F** – epithelial blastula; **G** – hatched swimming mesenchyme blastula, vegetal plate (vp) and mesenchyme cells (mc) are visible; **H** – early gastrula, ae – archenteron, bp – blastopore; **I** – mid-gastrula with extended narrow gastrocoele (gc), skeletogenous mesenchyme cells are visible on vegetal pole (mc); **J** – exogastrulation induced by incubation in hypotonic seawater; **K** – late gastrula, lateral view, archenteron grows in direction to vegetal side of the future larva, cell proliferation of the dorsal side of the future larva, the apical tuft is visible; **L** – coelomic cavities (c) grow from the archenteron, enterocoelic formation of the coelom; **M** – prism stage, mo – mouth, an – anus; sr – skeletal rods; **N** – echinopluteus, lc – left coelom; **O** – echinopluteus, ala – anteriolateral arm, poa – postoral arm; **P** – echinopluteus, skeletal rods (sr) and stomach with swallowed green algae are visible.



**ECHINODERMATA, ASTEROIDEA – *Asterias rubens***

Seastar *Asterias rubens* (Linnaeus, 1758) is a widespread species, readily available for collection in the tidal and subtidal zones. Seastars are dioecious, with spawning and external fertilization taking place in July. Gametes for artificial fertilization can be obtained from sexually mature females ready to spawn, starting from the end of June. To do this, dissection of the starfish arm should be conducted and sex established according to the colour of the gonads. Ovaries are orange (Fig. 34 A, B), while testes are whitish (Fig. 34 D, E). An ovary should be cut, wrapped in a piece of fine mesh (about 200 µm) (Fig. 34 C) and gently shaken in a cup with filtered seawater. The resulting suspension of eggs should be washed with filtered seawater and the maturity of eggs checked with a microscope. A large germinal vesicle is present in the immature oocyte (Fig. 35 A), while the cytoplasm of a mature egg is homogeneous (Fig. 35 B). Testes should be placed in a small amount of filtered seawater (Fig. 34 F) and the resulting suspension checked for sperm motility by microscopy. In vitro fertilization can be performed by adding a few drops of mobile sperm to a suspension of mature eggs.



**Figure 34. *Asterias rubens* gametes obtaining.**

**A** – dissected arm of female, fg – female gonad, lv – liver; **B** – orange ovary isolated from female; **C** – ovary placed on a piece of mesh for washing out oocytes; **D** – dissected arm of male, mg – male gonad, lv – liver; **E** – whitish testis isolated from male; **F** – sperm released from the testis.

A fertilization envelope and polar bodies will appear soon after fertilization (Fig. 35 C, D). Cleavage of starfish is holoblastic; the first two cleavage furrows are meridional and divide the zygote into four equal blastomeres (Fig. 35 E, F). Subsequent cleavage occurs in accordance with equal radial type. Morula is formed within six hours after fertilization and a blastocoel appear later (Fig. 35 G). The epithelial cilia-covered blastula is formed once 20 hours have passed after fertilization (Fig. 35 H). A vegetative plate appears and primary mesenchyme cells begin to immigrate to blastocoel by the time of hatching (Fig. 35 I).

Gastrulation occurs about 30-40 hours after fertilization. A tubular archenteron forms as a result of invagination of the blastula vegetative wall (Fig. 35 J). During gastrulation, the archenteron is lengthened and deviates to the ventral wall of the larva (Fig. 35 K). A pair of coelomic pouches is separated from the archenteron at later stages of development (Fig. 35 L). Early bilaterally symmetrical larva called dipleurula forms (Fig. 35 M-P). A mouth breaks through at the site of contact between the top of the gastrocoel and the ventral ectoderm. The gastrocoel is differentiated into the throat, stomach and hindgut, while the blastopore becomes the anus (Fig. 35 N).



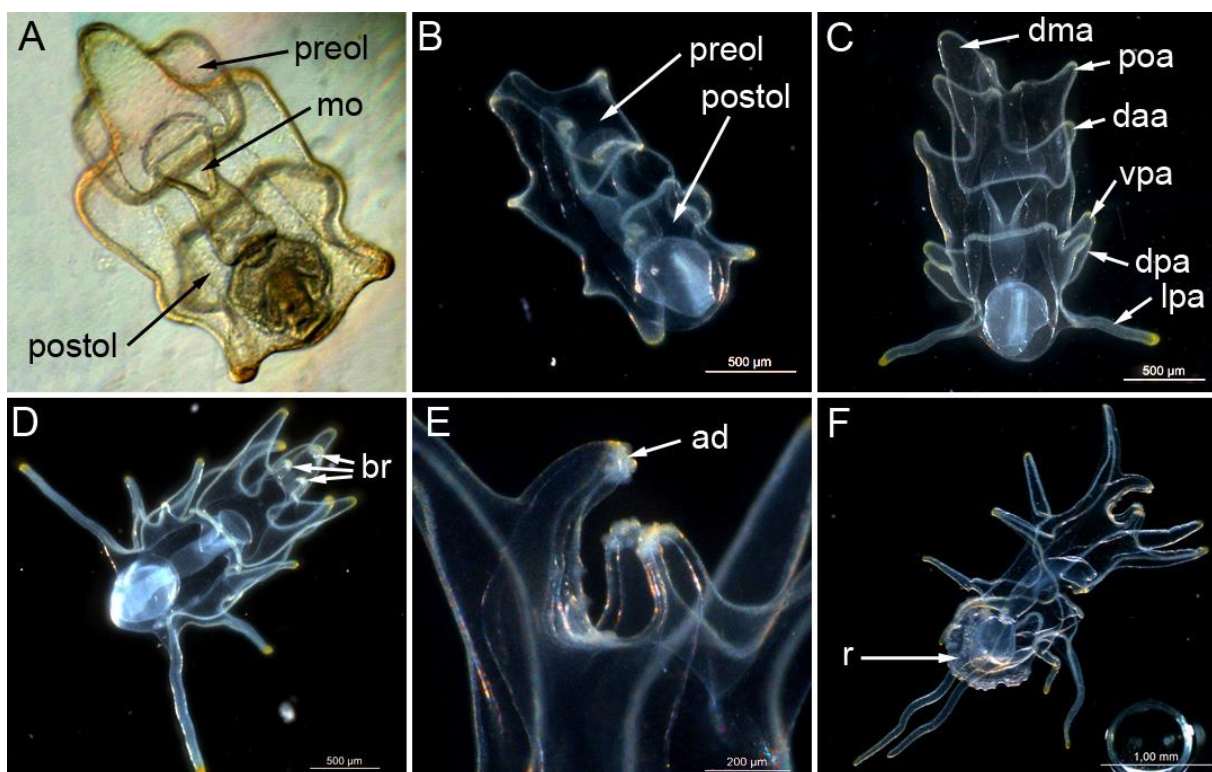
**Figure 35. Development of *Asterias rubens*.**

**A** – immature oocyte with germinal vesicle (gv); **B** – mature egg; **C** – fertilized egg with fertilization envelope (fe); **D** – zygote with polar body (pb); **E** – 2-cell embryo; **F** – 4-cell embryo; **G** – early blastula, bc – blastocoel; **H** – epithelial blastula, bc – blastocoel; **I** – hatched floating mesenchyme blastula, vp – vegetal plate; **J** – early gastrula, mesenchymal cells (mc) are visible in blastocoel; **K** – late gastrula, long narrow archenteron (ae) is curved in dorso-ventral plane, bc – blastocoel, gc – gastrocoel, bp – blastopore; **L** – enterocoel formation of coelomic pouches (c); **M** – dipleurula in frontal view, differences in the size of the left (lc) and right (rc) coeloms are visible; **N** – dipleurula in side view, digestive tube is differentiated into throat (ph), stomach (st) and hindgut (hg), mouth (mo) is broken through on the ventral side, anus (an) is formed from the blastopore; **O** – early bipinnaria in the frontal projection; **P** – early bipinnaria in side view, preoral (preol) and postoral (postol) lobes are visible. Scale bar 50 µm.



In later stages of development, the left-right asymmetry of coeloms occurs and ciliated bands appear in the ectoderm of larva, now called bipinnaria (Fig. 36 A, B). When viewed from the side, it can be seen that the dorsal side of bipinnaria is actively expanded during its development and as a result, the gut is curved and the mouth and anus are on the ventral side (Fig. 35 N, P; 36 B). The preoral lobe is formed anteriorly of the mouth, while the anus is on the postoral lobe (Fig. 35 P; 36 A, B). Ciliated bands appear at the edges of the blades and expand as outgrowths, called arms (Fig. 36 C). Coelomic pouches grow and merge into a single horseshoe-shaped sac that surrounds the throat and stomach.

Brachiolaria larva form roughly a month following fertilization. Brachiolar arms carrying the adhesive disks appear on the preoral lobe (Fig. 36 D, E). Rudiments of the radial channels are formed from the left hydrocoel, while mesenchymal cells start to form the skeletal elements of the future starfish (Fig. 36 F). A juvenile starfish arises from this area of the larva during the subsequent metamorphosis.



**Figure 36. Larvae and metamorphosis of *Asterias rubens*.**

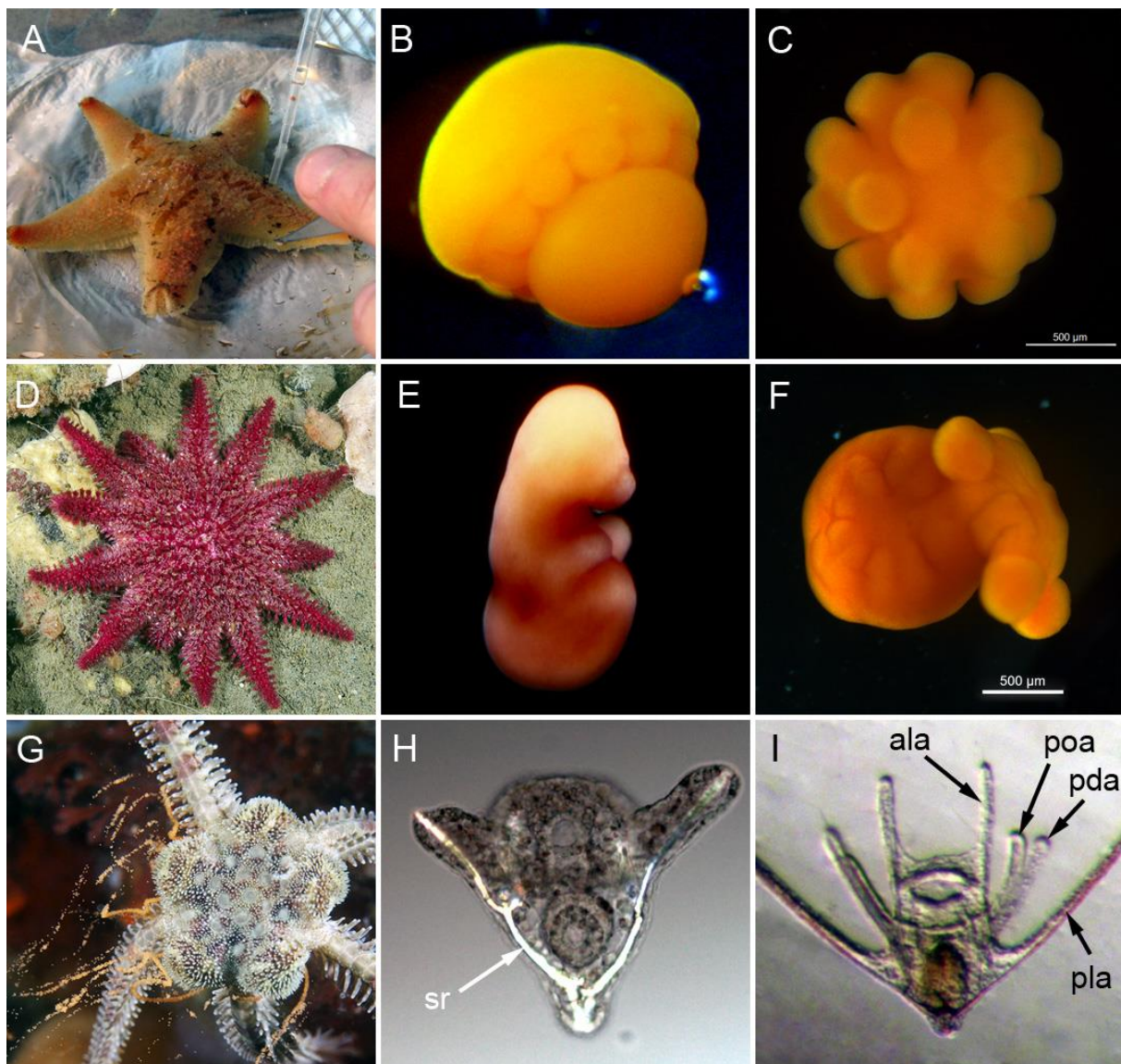
**A-B** – bipinnaria, preol – preoral lobe, postol – postoral lobe, mo – mouth; **C-D** – brachiolaria with 11 larval arms: unpaired dorso median one (dma), paired preoral (poa), paired dorsoanterior (daa), paired ventroposterior (vpa), paired dorsoposterior (dpa), paired lateroposterior (lpa) ones, brachiolarian arms (br); **E** – brachiolarian arms each ending in an adhesive disc (ad); **F** – late brachiolaria with adult rudiment (r).

**ECHINODERMATA****ASTEROIDEA**

Most White Sea starfish have polylecital eggs and direct development. Embryos of *Pteraster militaris* (Muller, 1776) develop in the subdorsal cavity of females (Fig. 37 A-C), where optimal conditions are provided by aeration. Formed juvenile starfishes leave the brood pouch through breaks in the membrane. Eggs and lecithotrophic larvae of *Crossaster papposus* (Linnaeus, 1768) (Fig. 37 E) are found in plankton during March.

**OPHIUROIDEA**

Brittle stars *Ophiopholis aculeata* (Linnaeus, 1767) and *Ophiura robusta* (Ayres, 1851) spawn eggs and sperm during June-July (Fig. 37 G). Ophiopluteus larvae are common in plankton samples at the beginning of the summer. They differ from the echinopluteus by the more obtuse angle present between the postoral arms (Fig. 37 H). During development, the postoral arms of ophiopluteus become extremely long (Fig. 37 I) and rudiment is formed; these become a brittle star body after metamorphosis.



**Figure 37. Development of White Sea echinoderms.**

**A** – *Pteraster militaris* embryos obtaining from the subdorsal cavity of the female; **B-C** – *P. militaris* lecithotrophic larvae (mesogens); **D** – *Crossaster papposus* adult; **E** – *C. papposus* lecithotrophic larva; **F** – *Henricia* sp. Lecithotrophic larva; **G** – spawning *Ophiopholis aculeata* female; **H** – ophiopluteus, skeletal rods (sr) are visible; **I** – late ophiopluteus, ala – anteriolateral arm, poa – postoral arm, pda – posterodorsal arm, pla – posterolateral arm.

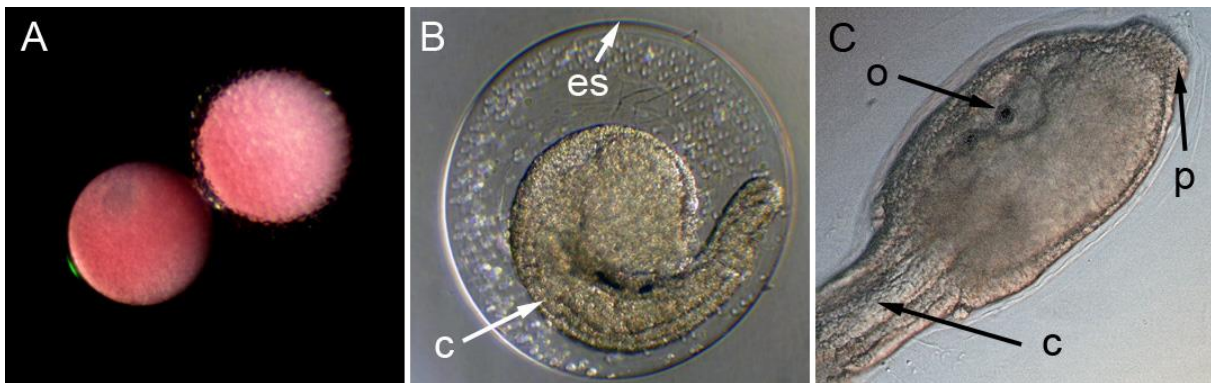


**CHORDATA (TUNICATA)****ASCIDIACEA**

Several species of single sea squirts are available in the White Sea: *Styela rustica* (Linnaeus, 1767), *Boltenia echinata* (Linnaeus, 1767), *Halocynthia pyriformis* (Rathke, 1806) (Fig. 38), *Molgula griffithsii* (MacLeay, 1825). The breeding season of these ascidians is during July-August. Developing embryos and larvae can be obtained by dissection of an adult sea squirt and washing its mantle cavity. Radial holoblastic cleavage, gastrulation by invagination and the formation of “tadpole” larva can be observed. The larva is folded inside the egg membrane prior to hatching (Fig. 39 B). Notochord, brain bubble, a light-sensitive eye and otolith are clearly distinguishable in the hatched larva (Fig. 39 C). The larva has gill slits in the throat, which lead into the peribranchial cavity and form the gill basket. There are papillae at the front end of the larvae, via which it is attached to the substrate during metamorphosis. Degeneration of the notochord and tail muscles of the neural tube occurs during metamorphosis.



**Figure 38. *Halocynthia pyriformis* adult.**



**Figure 39. Development of White Sea ascidians.**

**A** – *Molgula retortiformis* (Verrill, 1871) oocytes; **B** – *Halocynthia pyriformis* larva in the egg shell, c – chord; **C** – *H. pyriformis* larva's head o – otolith; p – papilla; c – chord.

**APPENDICULARIA**

*Oikopleura vanhoeffeni* (Lohmann, 1896) is the only appendicularian species in the White Sea. Adults appendicularians are hermaphrodites. Larvae and juveniles are often found in plankton at the beginning of summer (Fig. 40).



**Figure 40. *Oikopleura vanhoeffeni* larva.**

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