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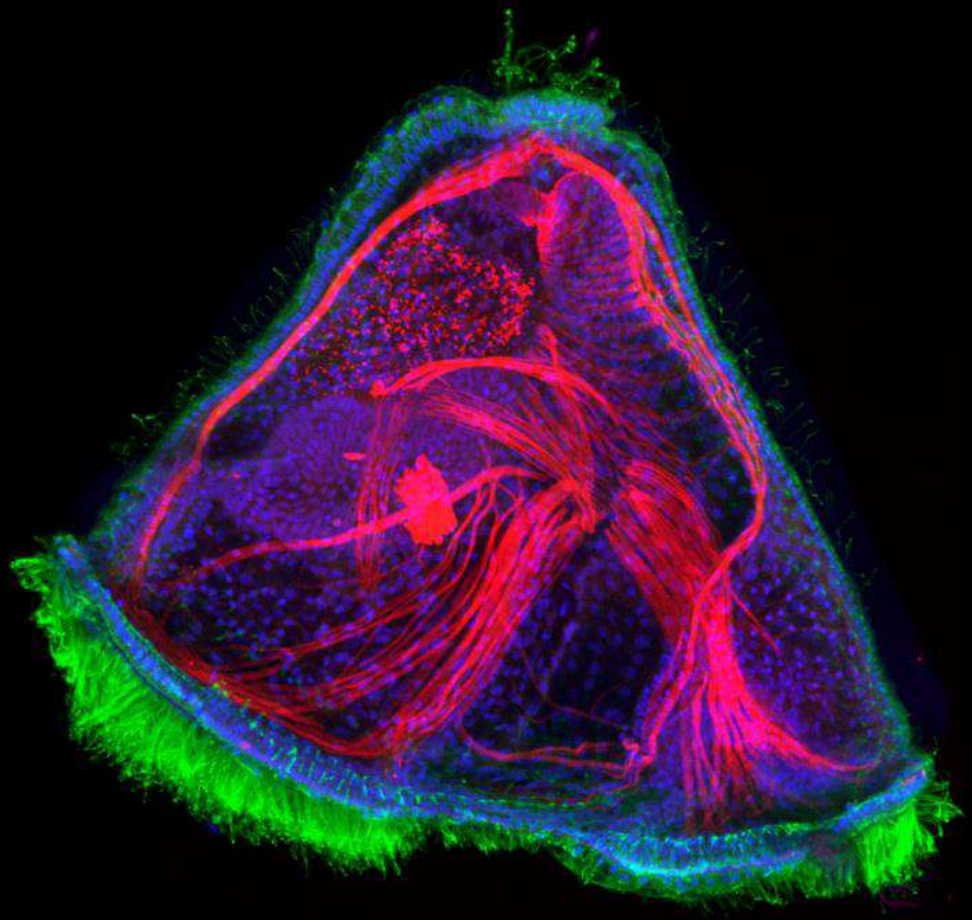

WSBS MSU
2019

Atlas of the White Sea Invertebrates Development

3rd Summer Course on Embryology
of Marine Invertebrates

June 9-30, 2019

WSBS MSU, Russia

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This Atlas contains a summary of the main invertebrate animals available for studying their embryology and development processes at the Pertzov White Sea Biological Station (WSBS) MSU, Russia. The illustrations used in the Atlas are collected thanks to the efforts of a large team of people working at the WSBS MSU. The release of the Atlas is timed to the 3rd Summer Course on Embryology of Marine Invertebrates, which took place June 9-30, 2019 at the WSBS MSU. Detailed information can be found on the website of the biostation: http://en.wsbs-msu.ru

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PORIFERA

Several species of sponges are available for developmental studies at the beginning of summer. One of them, *Halisarca dujardini* (Johnston, 1842), is common on rhizoids of brown algae and has an irregular body shape with a smooth mucous surface (Fig. 1 A). There are many matured oocytes and embryos inside the sponge at the end of June (Fig. 2 E). Individual embryos at various developmental stages can be obtained using dissecting needles. 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 F) and an aquiferous system is regenerated during the next several days (Fig. 2 G-H).

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

Several species of calcareous sponges live in the White Sea (Fig. 1 C, D), but their reproduction mainly occurs in late autumn and winter. For example, *Leucosolenia complicata* (Montagu, 1812) amphiblastula larvae can be found in January (Fig. 2 K). Representatives of the genus *Sycon* (Risso, 1826) live at a depth of 1.5 m among the red algae and kelp. They have elongated body with single osculum surrounded by a corolla of long calcareous spicules (Fig. 1 D). In the White Sea reproduction of *Sycon* occurs in winter, but it is an excellent object for the study of regeneration in any season.

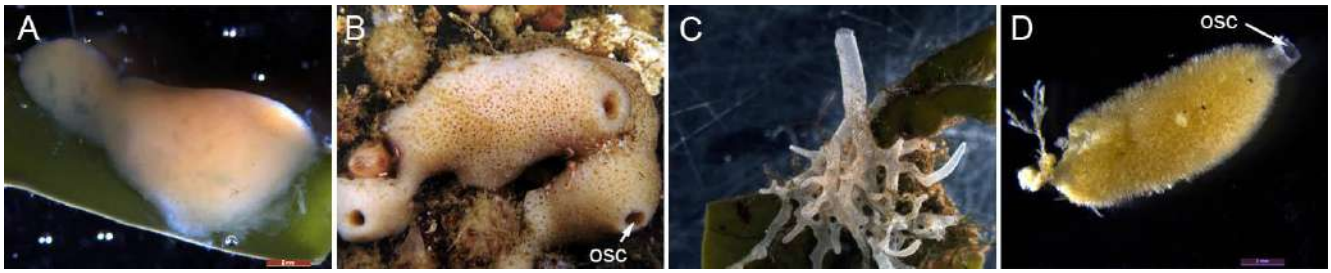

Figure 1. White Sea sponges.
A – *Halisarca dujardini* on the broun alga tallom; **B** – *Haliclona aquaeductus* with several osculumes (osc); **C** – *Leucosolenia complicata*; **D** – *Sycon sp.*, osc – osculum.

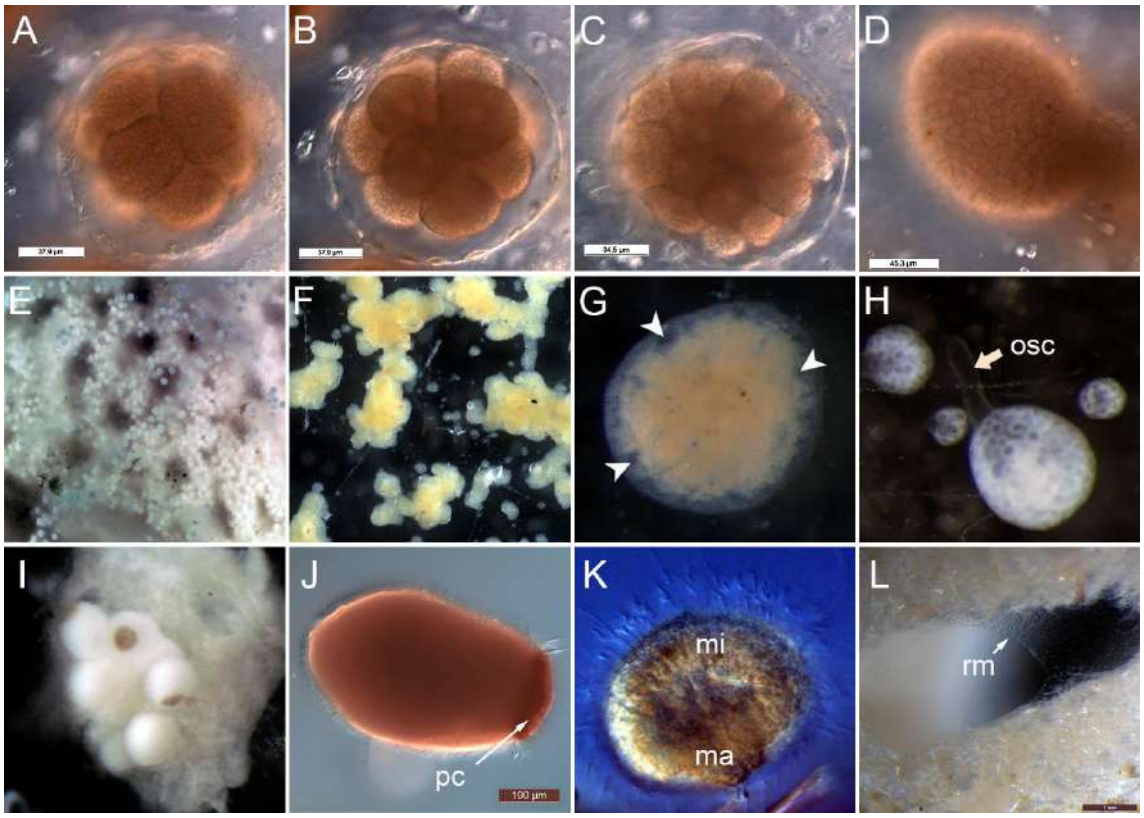

Figure 2. Development of White Sea sponges.
A-D – *Halisarca dujardini* embryonic development; **A-C** – embryos at the cleavage stage (16, 32 and 64 cells); **D** – blastula; **E** – *H. dujardini* embryos inside the sponge; **F-H** – reaggregation of dissociated *H. dujardini* cells; **F** – cell aggregates 2 h after dissociation; **G** – attached primmorph with formed aquiferous system cavities (arrowheads); **H** – regenerated sponge with formed osculum (osc); **I** – *Haliclona aquaeductus* parenchymulae inside the sponge, **J** – *H. aquaeductus* parenchymula, pc – pigmented cells, **K** – *Leucosolenia complicata* amphiblastula larva, ma – macromeres, mi – ciliated micromeres; **L** – formation of regenerative membrane in transversely sectioned *Sycon sp*. body.

CNIDARIA

HYDROZOA

The majority of hydrozoans possess metagenetic life cycle with alternation of life forms (or stages): a sedentary juvenile stage (polypoid colony) asexually produces an adult stage (medusa); the medusa produces gametes, which fuse and the resulting embryo develops into a planula larva; the planula metamorphoses into a sessile primary polyp, the founder of a new colony. However, the reduction of life cycle stages (hypogenesis) occurs quite frequently. *Aglantha digitale,* which has no polyp stage (Fig. 4) is one such example. At the same time, in *Gonothyraea loveni,* medusa stage is reduced and attached medusoids are formed instead of free-floating medusa (Fig. 6). Unlike medusae, medusoids have no mouth opening, but they have radial channels, small tentacles and closed subumbrellar cavity. *Coryne lovenii* (M. Sars, 1846) exhibits the same stage of medusa reduction.

Clava multicornis (Forskal, 1775) forms stolonial colonies consistng of creeping stolons and the hydrants budding from stolons. Mutiple gonophores representing the reduced medusae sit tightly on the hydrants forming the belt right under the tentacles (Fig. 3 E). The rudiment of subumbrella cavity is the only remaining feature of the medusa. Embryos of *C. multicornis* develop inside the female gonophores in August. They leave gonophores only at the planula larva stage. Gonophores are also formed on the hydrants of *Ectopleura (Tubularia) larynx* (Ellis & Solander, 1786). *Ectopleura* exhibits direct development and actinula larvae with tentacles (instead of planula larvae) are formed in gonophores in July and August.

The next stage of medusa reduction is observed in *Dynamena pumila* (Linnaeus, 1758) and *Laomedea flexuosa* (Alder, 1857). Two layered sacs with no medusa structres (sporocacs) are formed on the sexual zooids (gonozooids) sitting inside the gonothecae in July (Fig. 8 A, B).

Many White Sea hydrozoans have a complex life cycle with free-floating medusa stage. Numerous small flat medusae (Fig. 3 D) of *Obelia longissima* (Pallas, 1766) appear in plankton in June and of *Obelia geniculata* (Linnaeus, 1758) in August. Four gonads are formed on the medusa radial channeles. Medusae of *Sarsia tubulosa* (M. Sars, 1835) (Fig. 3 C), *Bougainvillia superciliaris* (Agassiz, 1849) (Fig. 3 A) and *Rathkea octopunctata* (M. Sars, 1835) are common in plankton in June.

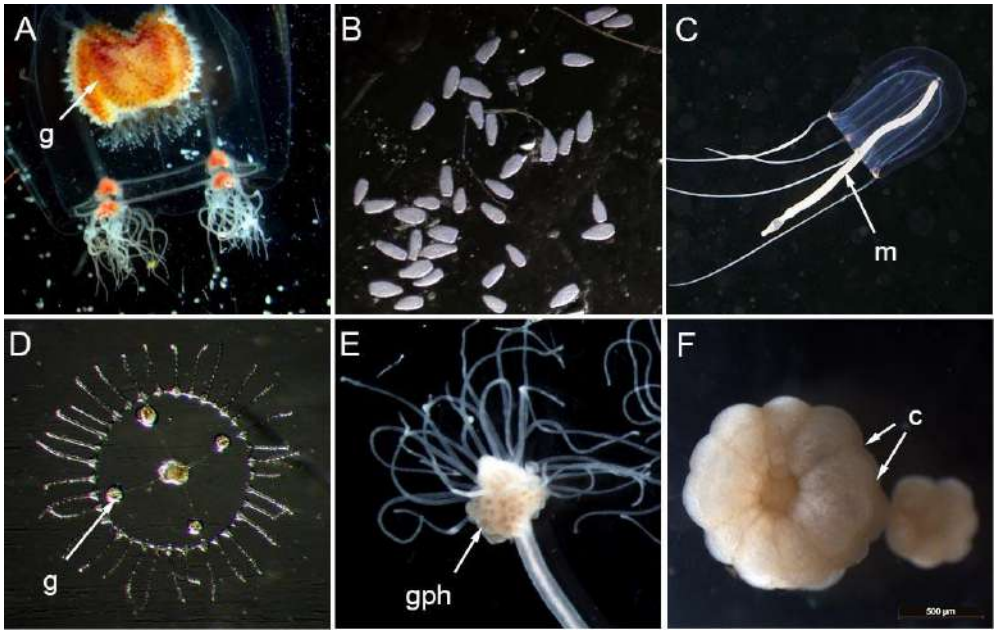

Figure 3. Development of White Sea cnidarians.
A – *Bougainvillia superciliaris* medusa, orange gonads (g) containing embryos and planulae are are visible on the manubrium; **B** – *B. superciliaris* planulae; **C** – *Sarsia tubulosa* medusa, long manubrium (m) contains gonads © Alexander Semenov; **D** – medusa of *Obelia longissima* with four gonads (g); **E** – *Clava multicornis* with gonophores (gph); **F** – juveniles of the antozoan *Aulactinia stella* taken from the parent anemone, constrictions (c) coincide with the developing septae.

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 red tentacles around the edge (Fig. 4). 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 gonad content. In the White Sea, their reproduction takes place from mid-June to August. Mature gametes are spawned into the water column. 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.

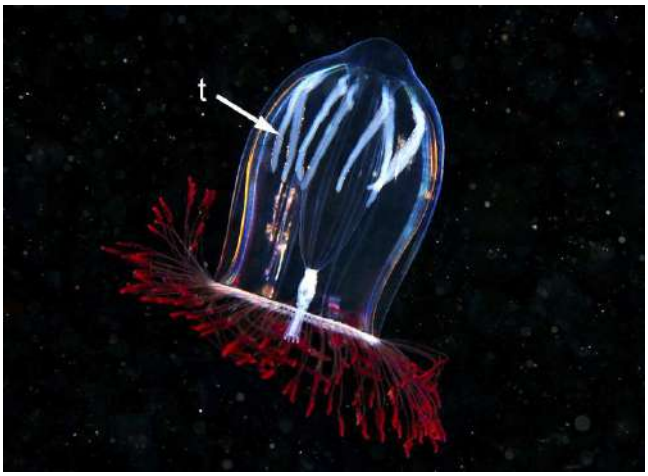

Figure 4. Adult male of *Aglantha digitale*.
Eight testes (t) are visible in the upper part of the bell. © Alexander Semenov

Eggs of *A. digitale* (Fig. 5 A) are rich in yolk granles. In the course of holoblastic cleavage morula is formed (Fig. 5 B, C). Then gastrlation by delamination (segregation of the ectoderm from the endoderm) takes place (Fig. 5 D). A planula covered with the cilia (Fig. 5 E, F) does not settle, but transforms at first into an actinuloid larva (Fig. 5 G, H) and then into a young medusa.

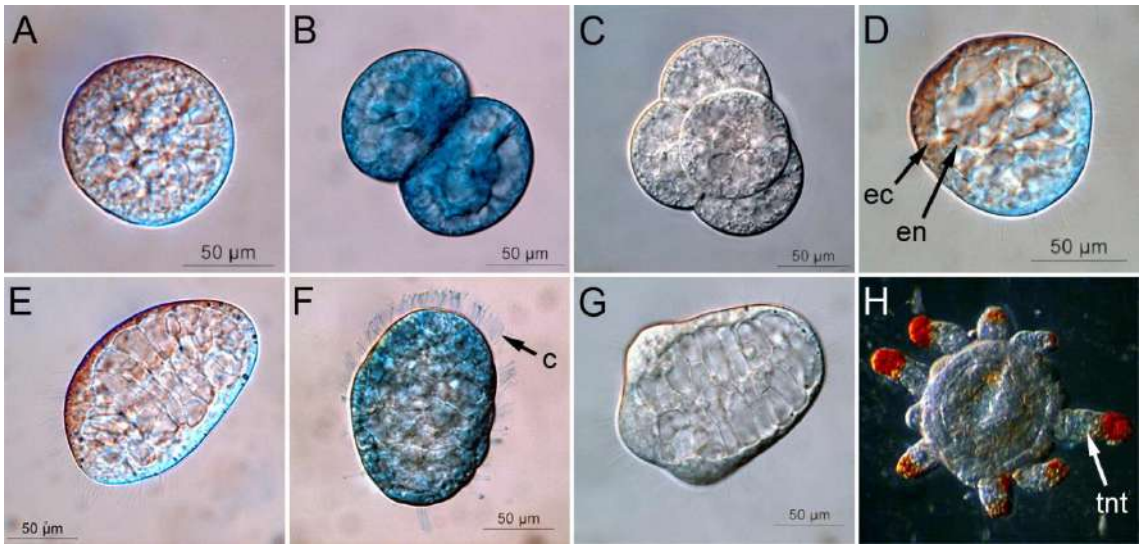

Figure 5. Development of *Aglantha digitale*.
A – zygote; **B** – 2-cell embryo; **C** – 4-cell embryo demonstrating a tetrahedral packing of the blastomeres; **D** – gastrulation by delamination, ec – ectoderm, en - entoderm; **E** – planula larva; **F** – planula stained with Janus Green, cilia (c) are visible; **G** –rocket-like late planula larva specific for *Aglantha*; **H** – actinuloid larva with tentacles (tnt) which later develops into a young medusa.

Gonothyraea loveni

Colonies of *Gonothyraea 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 containing gonozooids with maturing medusoids appear in colonies (Fig. 6 A). Mature medusoids leave the gonothecae, but remain attached to gonozooids (Fig. 6 A-C). Colonies of *G. loveni* are dioecious; 2-4 eggs are located in each female medusoid (Fig. 6 A, C). Fertilization and embryonic development occurs inside the female medusoids.

To observe embryonic development, individual embryos need to be removed from the medusoid using dissecting needles. Note that all embryos in medusoid are at the same stage of development.

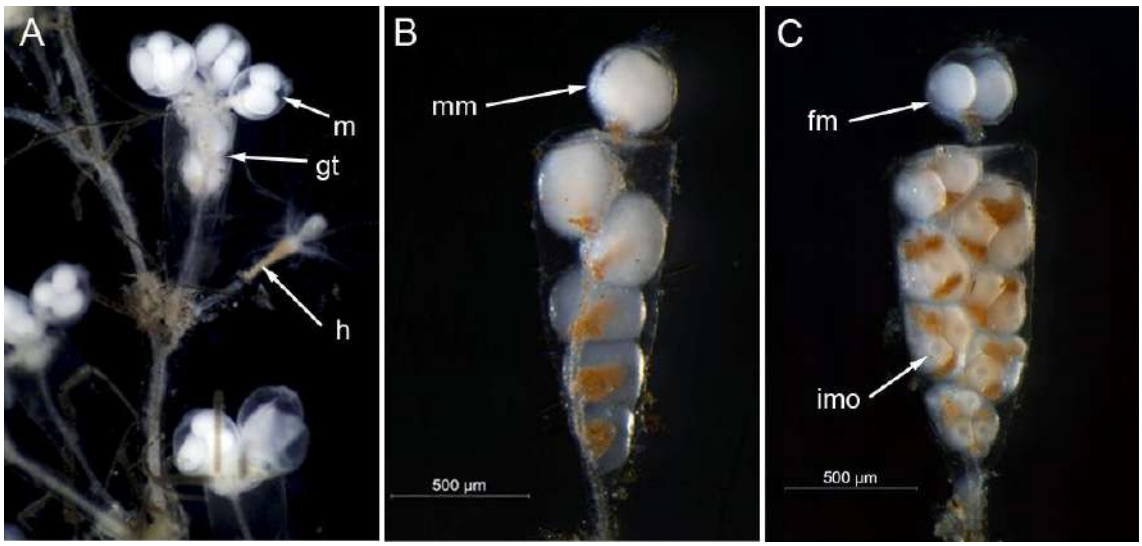

Figure 6. Colonies of *Gonothyraea loveni*.
A – colony fragment with hydrant (h) and female gonothecae (gt) with extruded mature medusoids (m); **B** –gonotheca with extruded male medusoid (mm); **C** – female gonotheca with extruded mature medusoid (fm) and medusoids with immature oocytes (imo). © Yulia Burmistrova

Cleavages in *G. loveni* are all complete and holoblastic. This species exhibits unilateral cleavage furrows typical for cnidarians. The cleavage furrow initiates at one pole of the zygote or a blastomere and spreads towards the opposite pole (Fig. 7 C, G). Starting from the second round of cleavage, active movements of the blastomeres provide their compact packing resembling the pattern of spiral cleavage observed in Spiralians. During late cleavage, it is impossible to find any regularity in cleavage divisions; thus, cleavage becomes irregular. This irregular cleavage produces a morula (Fig. 7 I-J), and gastrulation in *G. loveni* occurs by morular delamination (Fig. 7 K).

Embryos develop into the planula larvae (Fig. 7 M) that leave medusoids. The planula is a typical larval stage of cnidarians. The hydrozoan planula is uniformly ciliated, has an oval shape, usually opaque and lacks gut (it does not feed). Hydrozoan planulae usually spend a short period of time (several days or even houres) in plankton, then settle (Fig. 7 N) and undergo metamorphosis transforming into a benthic polyp (Fig. 7 O, P). Planulae kept in a Petri dish with seawater settle in 1-2 days.

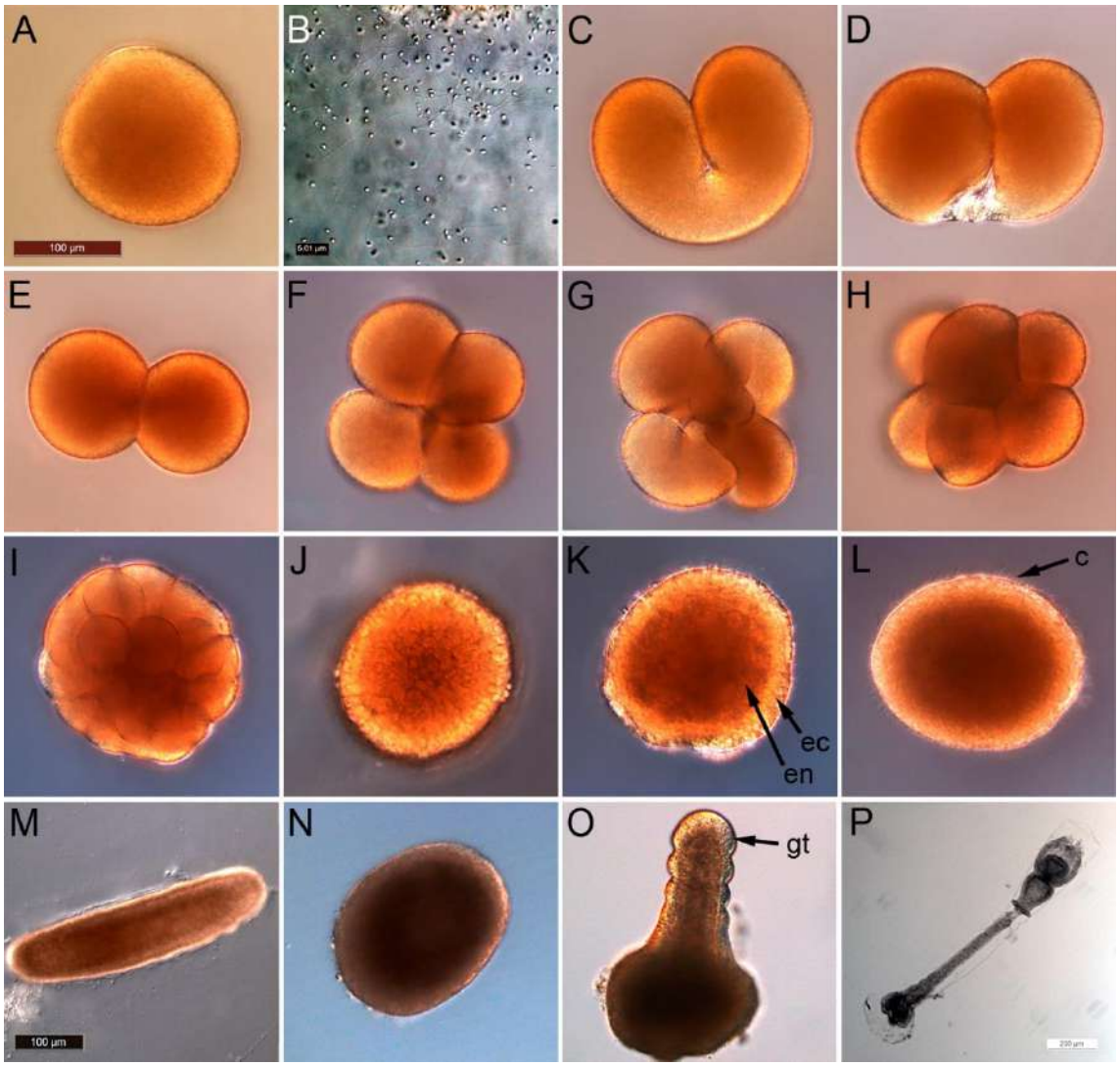

Figure 7. Embryonic development and metamorphosis of *Gonothyraea loveni*.
A – zygote; **B** – sperm cells isolated from the male medusoid; **C** – the first unilateral cleavage furrow; **D** – end of the first cleavage; **E** – 2-cell embryo; **F** – 4-cell embryo; **G** – the third cleavage stage, unilateral cleavage furrows are visible; **H** – 8-cell embryo demonstrating the pseudo-spiral cleavage pattern; **I** – early morula; **J** – late morula; **K** – gastrula, ectoderm (ec) and entoderm (en) are already distinguishable; **L** – preplanula stage, cilia (c) are visible on its surface; **M** – planula larva; **N** – settled planula; **O** – formation of growing tip (gt) of the primary polyp; **P** – completely formed primary polyp. © Yulia Burmistrova (A-L), Elena Parshina, Natalia Sokolova (M-P).

Dynamena pumila

Colonies of *Dynamena pumila* (Linnaeus, 1758) form thickets on brown algae in the lower tidal zone and upper subtidal zone. Colonies of *D. pumila* are dioecious. Reproduction takes place in July, when gonozooids sitting in gonothecae form sporosacs containing gamets (Fig. 8 A, B). From 4 to 12 mature egg cells are extruded from female gonotheca within a mucous acrocyst, where the embryonic development tekes place (Fig. 8 D-K).

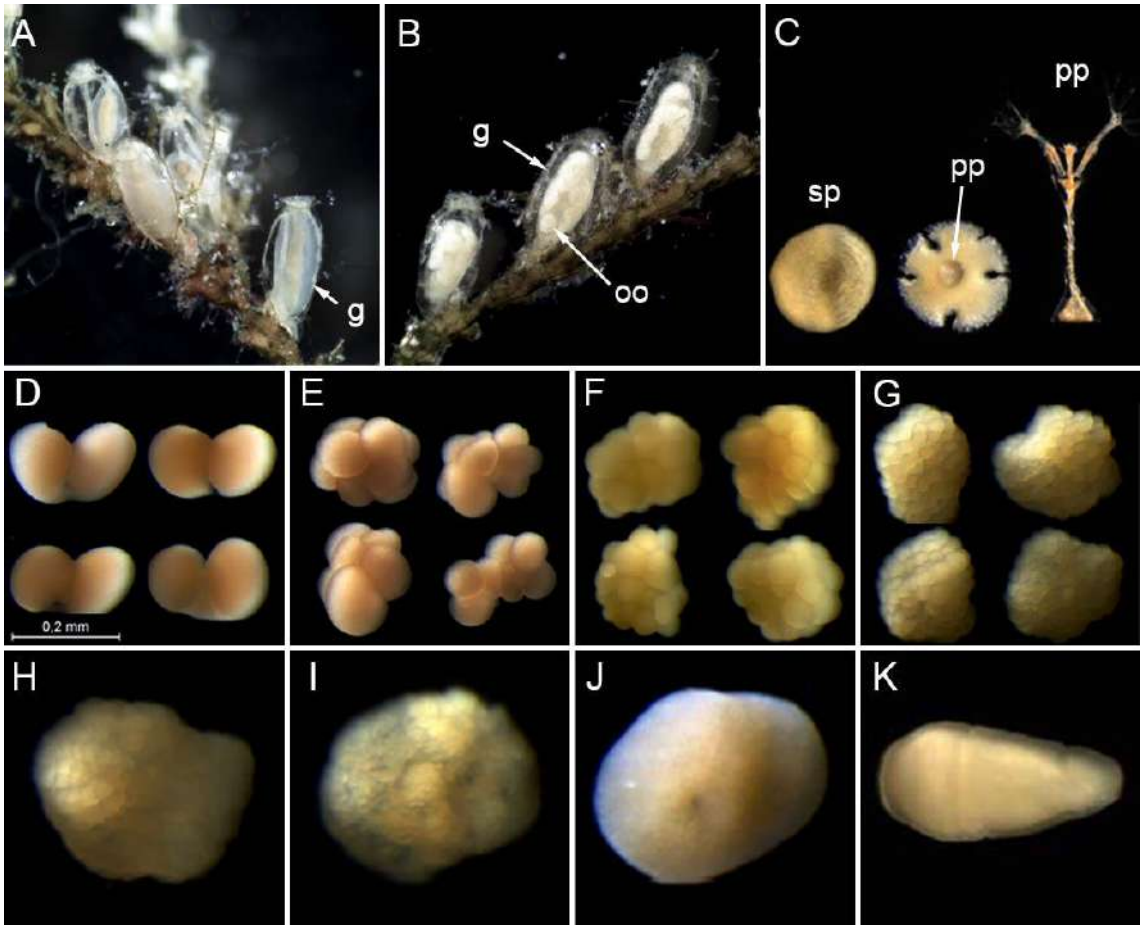

Figure 8. Embryonic development and metamorphosis of *Dynamena pumila*.
A – gonozooids (g) with male sporosacs inside the gonotheca; **B** – gonozooids (g) with female sporosacs, oocytes (oo) are visible; **C** – metamorphosis of planula larva: settled planula (sp) and two stages of primary polyp (pp) formation; **D-K** – embryonic development: **D** – 2-cell embryos, the first cleavage furrow is unilateral; **E** – 8-cell embryos; **F** – early morula; **G** – late morula; **H-J** – gastrulation **K** – planula.

SCYPHOZOA

The White Sea scyphozoans *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 and mouth lobs in the end of summer (Fig. 9 D-K). Settled planula form a polyp stage, scyphistoma (Fig 9 A), that reproduces asexually by budding. The ephyrae (Fig. 7 B, C) are formed during strobilation of scyphistomae. Ephyrae frequently occur in plankton in June and develop into adult jellyfishes by the end summer.

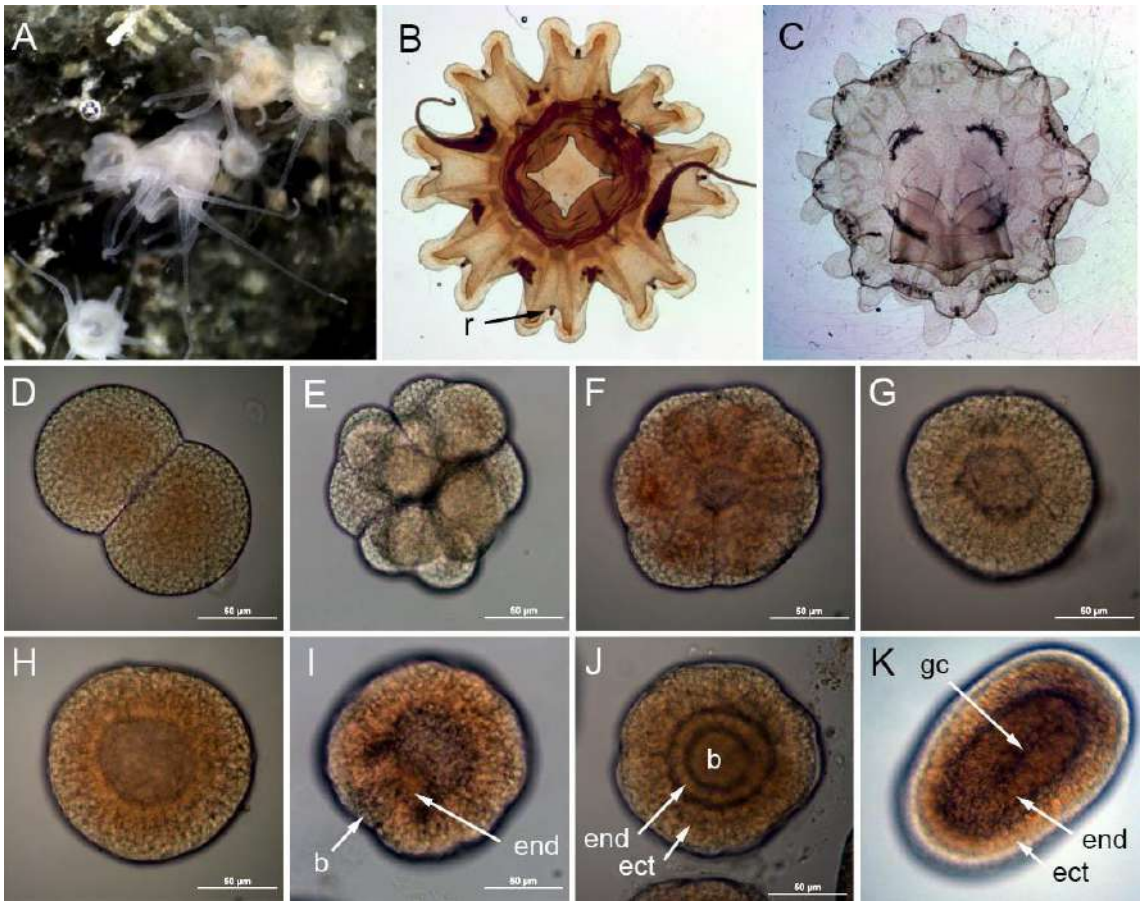

Figure 9. Development of White Sea scyphozoans.
A – sciphistomae; **B** – ephyra of *Cyanea*, each of the eight lobes contains rhopalium (r); **L** – ephyra of *Aurelia aurita*; **D-K** – embryonic development of *Aurelia*; **D** – 2-cells embryo; **E** – 16-cells embryo; **F** – early blastula; **G** – middle blastula; **H** – late blastula; **I** – gastrulation proceeding by invagination, b – blastopore, end – invaginating endoderm; **J** – gastrula, view from the blastopore (b), ect – ectoderm; end – endoderm; **K** – planula, ect – ectoderm; end – endoderm, gc - gastric cavity.

ANTHOZOA

Development of a sea anemone *Aulactinia stella* (Verrill, 1864) is direct (no planula larva stage) and occurs in mother's body. Using genetic analyses, Bocharova and Mugue (2012) showed that juveniles can also be bred in the non-maternal organism. Juveniles (Fig. 3 F) with developing septae can easily be found in the gastric cavity of adult anemones.

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).

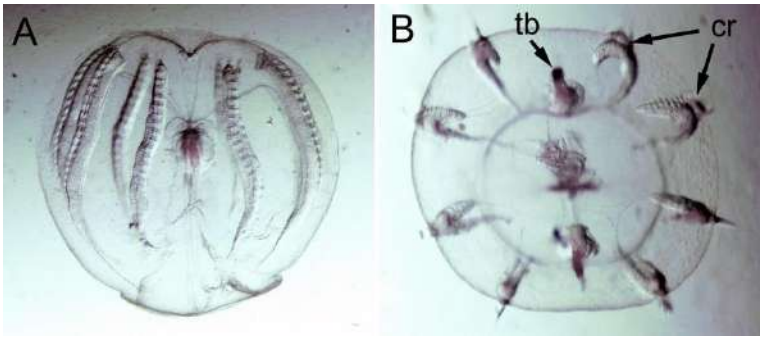

Figure 10. Ctenophora juvenile.
A – side view; **B** – view from the oral end, eight comb rows (cr) and tentacle bulbs (tb) are visible.

The embryonic development of all ctenophores is extremly uniform. Cleavage occurs by the forming of unilateral furrows (Fig. 11 A). The first and second cleavage furrows (Fig. 11 B) divide the egg according to the sagittal (pharingeal) and tentacular planes of the developing comb jelly. After the third cleavage, the embryo is divided into four central blastomeres M, and four lateral blastomeres E (Fig. 11 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. 11 D). Micromeres form an outer cell layer (ectoderm) that surrounds macromers (entoderm) (Fig. 11 E-G). This process can be considered as gastrulation by epiboly. Then macromeres are divided equally into 16 cells; 24 oral micromeres are formed and move deep into the gastric cavity (Fig. 11 F, G), where they form a cross-shaped primordium of tentacles muscles (Fig. 11 H). The cells of this primordium can be considered as mesodermal ones. Cydippid larva with eight comb rows and tentacles (Fig. 10) forms and hatches then.

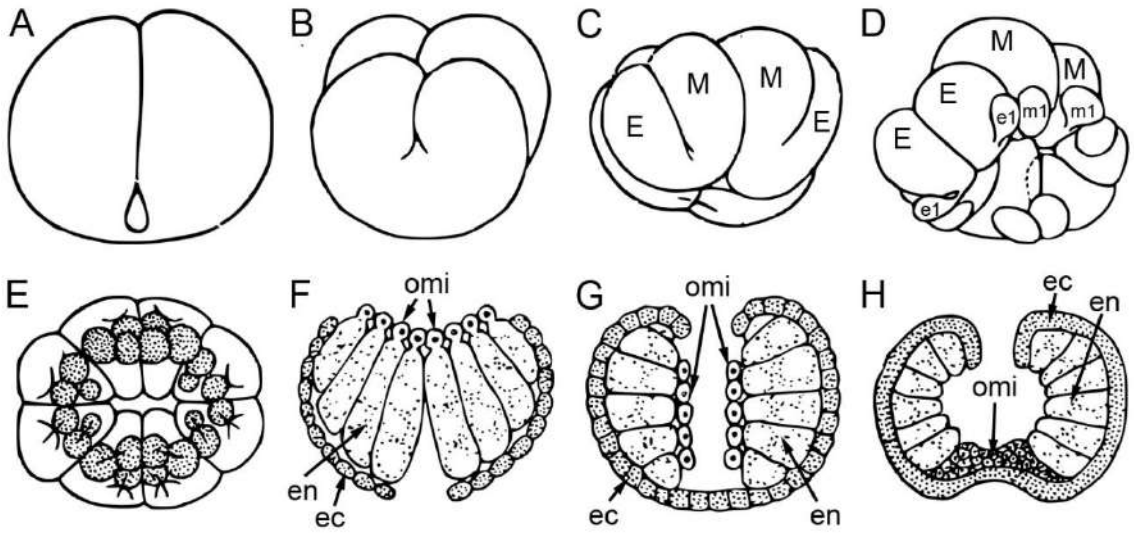

Figure 11. Embryonic development of ctenophores.
A-E – cleavage of *Beroe ovata*, © Ziegler, 1898; **A** – the first cleavage division by unilateral furrow formation; **B** – the second cleavage division; **C** – the third cleavage division, M and E blastomeres are formed; **D** – the fourth cleavage division, micromeres m1 and e1 are formed; **E** – cleavage embryo, aboral view; **F-H** – germ layers differentiation, lateral view, ec – ectoderm, en – entoderm, omi – oral micromeres © Davidov, 1914.

ANNELIDA

The vast majority of polychaetes have a planktonic larva stage during their life cycle (Fig. 12). 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 locates along the equator. In addition to prototroch, larva can have a few more ciliary belts – anal telotroch and intermediate mesotrochs (Fig. 12 C). An apical sense organ (ciliary tuft) is often 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. 12 G). In the nectochaete stage, internal segmentation and typical biramous parapodia appear in the larvae (Fig. 12 A, B, H, I). Nectochaetes acquire head appendages, which are typical for the adult worms and sink to the seabed to begin benthic life.

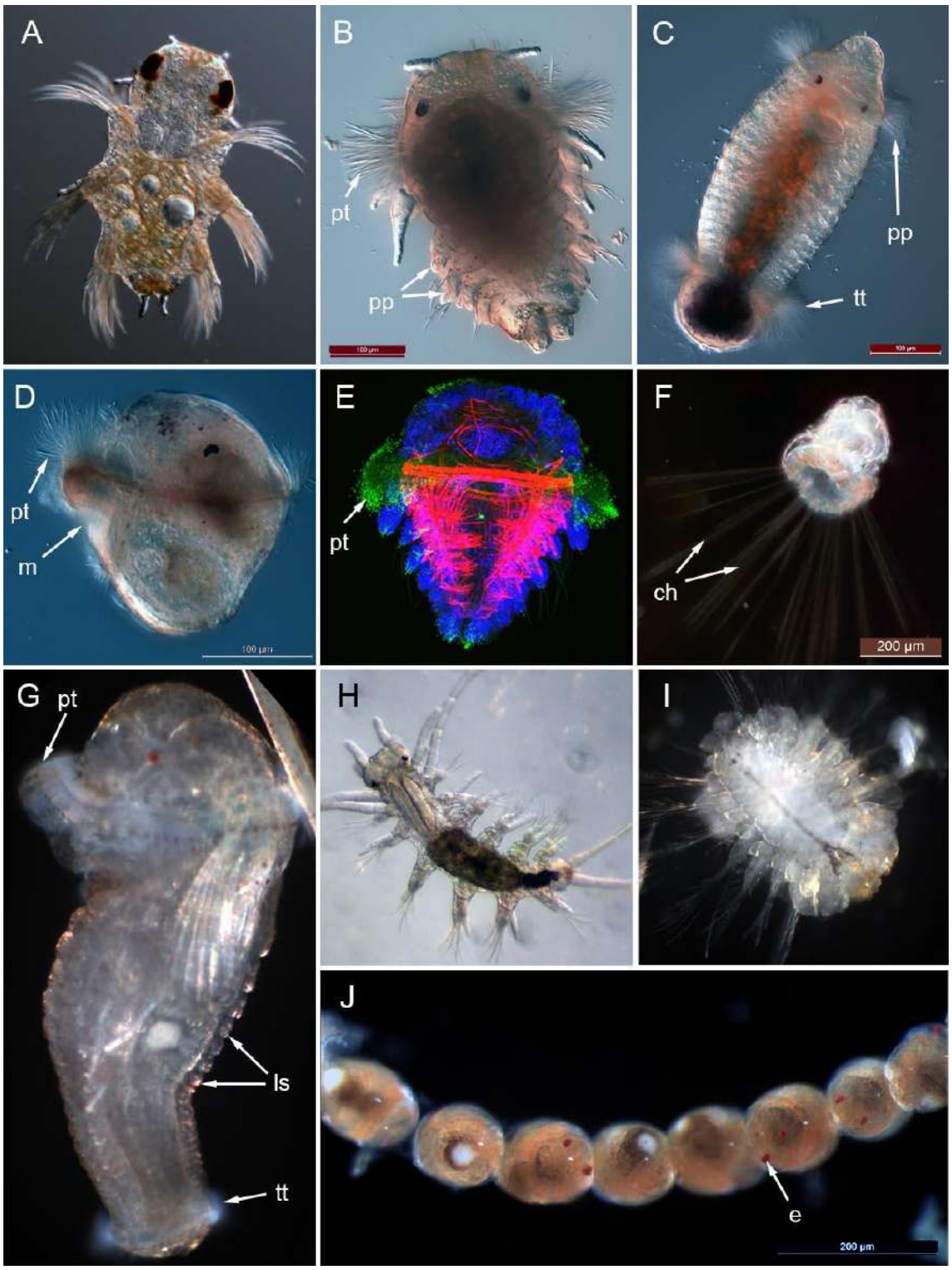

Figure 12. Trochophore larvae of White Sea polychaetes.
A – *Alitta (Nereis) virens* (Sars, 1835) nectochaeta with three larval segments; **B** – Phyllodocidae nectochaeta, pt – prototroch, pp - parapodia; **C** – *Capitella capitata* (Fabricius, 1780) metatrochophore, pt – prototroch, tt – telotroch; **D** – *Harmothoe imbricata* (Linnaeus, 1767) trochophore, pt – prototroch, m - mouth; **E** – Phyllodocidae nectochaeta fluorescent micrograph (nuclei are blue, cilia are green, actin filaments are red), pt – prototroch © Abraham Smith; **F** – *Galathowenia oculata* (Zachs, 1923) mitraria, ch – chaetae; **G** – *Pectinaria koreni* (Malmgren, 1866) nectochaeta, pt – prototroch, tt – telotroch, ls – larval segments; **H** – *Nereimyra (Castalia) punctata* (Muller, 1788) nectochaeta; **I** – *H. imbricata* nectochaeta, view from the ventral side, elytrae are visible behind the parapodia; **J** – chain of eggs of *Circeis armoricana* (Saint-Joseph, 1894) obtained from tube, trochophores with red eyes (e) are inside eggs.

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. 13). Individual eggs can be removed from the mucous capsules with a dissecting needle and cleavage can be observed with a microscope.

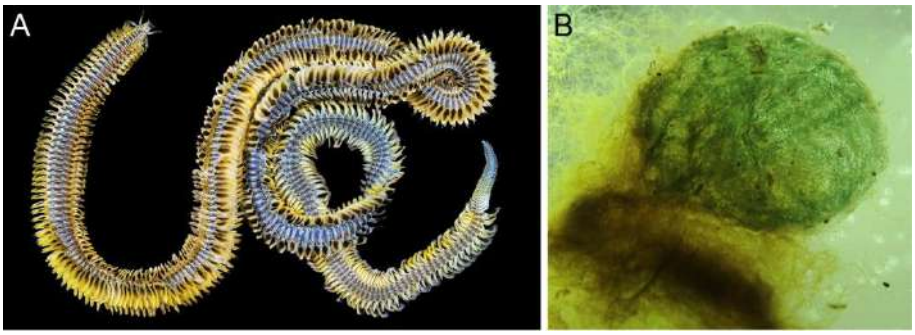

Figure 13. *Phyllodoce maculate* and its clutch.
A – adult worm © Alexander Semenov; **B** – green spherical mucous clutch attached to algae.

Like in all annelids, the eggs of *P. maculata* undergo spiral type cleavage (Fig. 14 A-D). D blastomere is usually larger than other blastomeres (Fig. 14 D). In general, after the first cleavage, CD blastomeres are bigger than AB (Fig. 14 C), but sometimes they are the same size (Fig. 14 B).

Trochophore larvae develop in the egg capsule (Fig 14 E, F). Trochophores have a prototroch and an apical organ (Fig 14 F). Two red eyes can be distinguished in an episphere (Fig 14 E). Larval segments and parapodia appear at later stages of free-floating metatrochophore and nectochaete (Fig. 14 G, H).

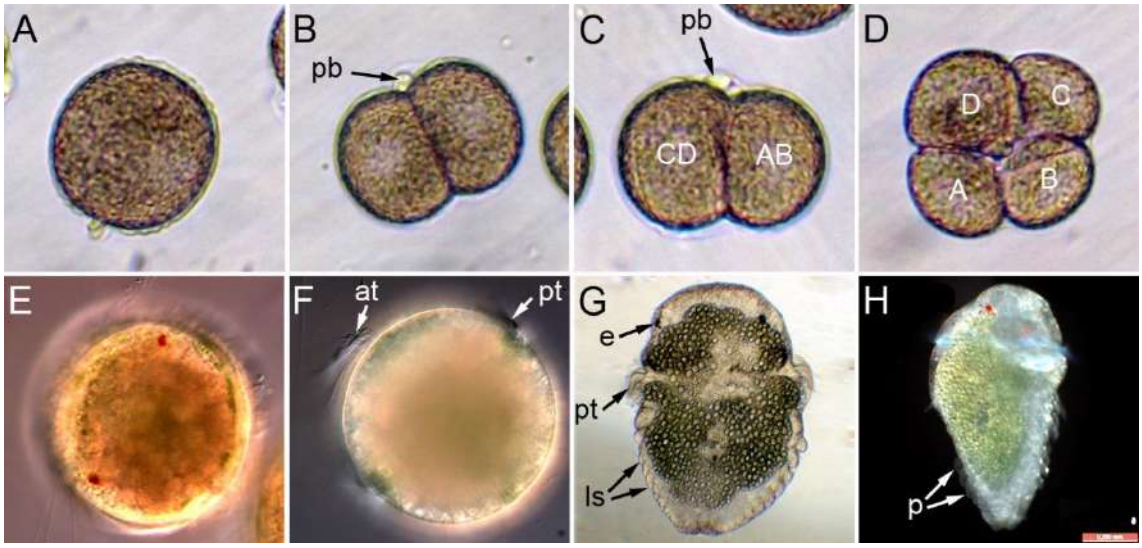

Figure 14. Development of *Phyllodoce maculata*.
A – zygote; **B** – 2-cell stage, blastomeres AB and CD are equal, pb – polar bodies; **C** – 2-cell stage, blastomere CD is slightly bigger than AB, pb – polar bodies; **D** – 4-cell stage, blastomer D is bigger than the others; **E** – trochophore, view 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.

Ophelia limacina

Annelid *Opelia 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. 15). 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.

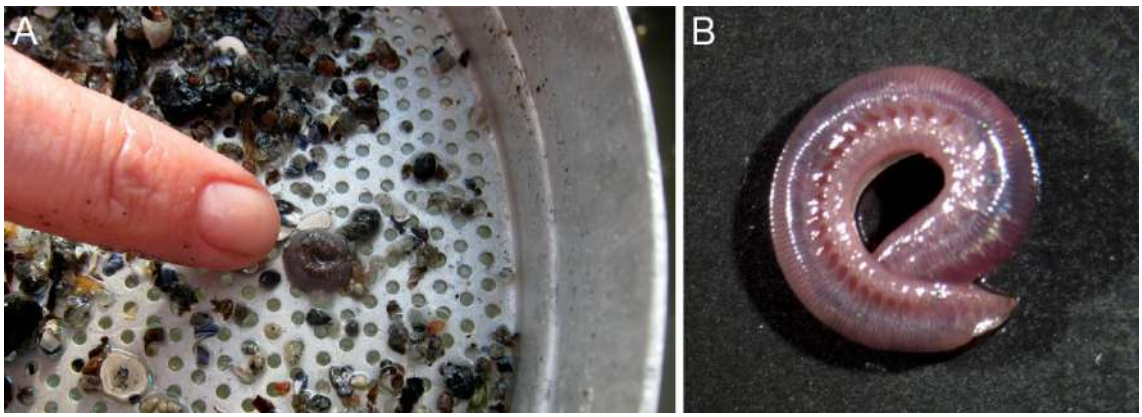

Figure 15. *Ophelia limacina*.
A – extraction the worms from the sediment with a sieve; **B** – rinsed adult worm.

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 16 A, B). As a result, the initially flat, lentil-shaped egg gains a spherical shape (Fig. 16 C). Two polar bodies form within a couple of hours following fertilization (Fig 16 D, E) and cleavage starts (Fig 16 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. 16 G). As a result of the third cleavage, a quartet of vegetative macromers (1A-1D) and a quartet of animal micromeres (1a-1d) appear (Fig 16 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 examined on molluscs.

The main quartet of blastomeres is visible as four large cells at the vegetal pole of the 64-cell embryo (Fig 16 J). These cells are immersed within the embryo (Fig. 16 K, L) and form the wide invagination (Fig. 16 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. 16 O). Stages of further larval development can be observed during the month following the fertilization (Fig. 16 P-S). 21 days-old nectochaetae begin settlement. The larvae should be fed by unicellular green algae.

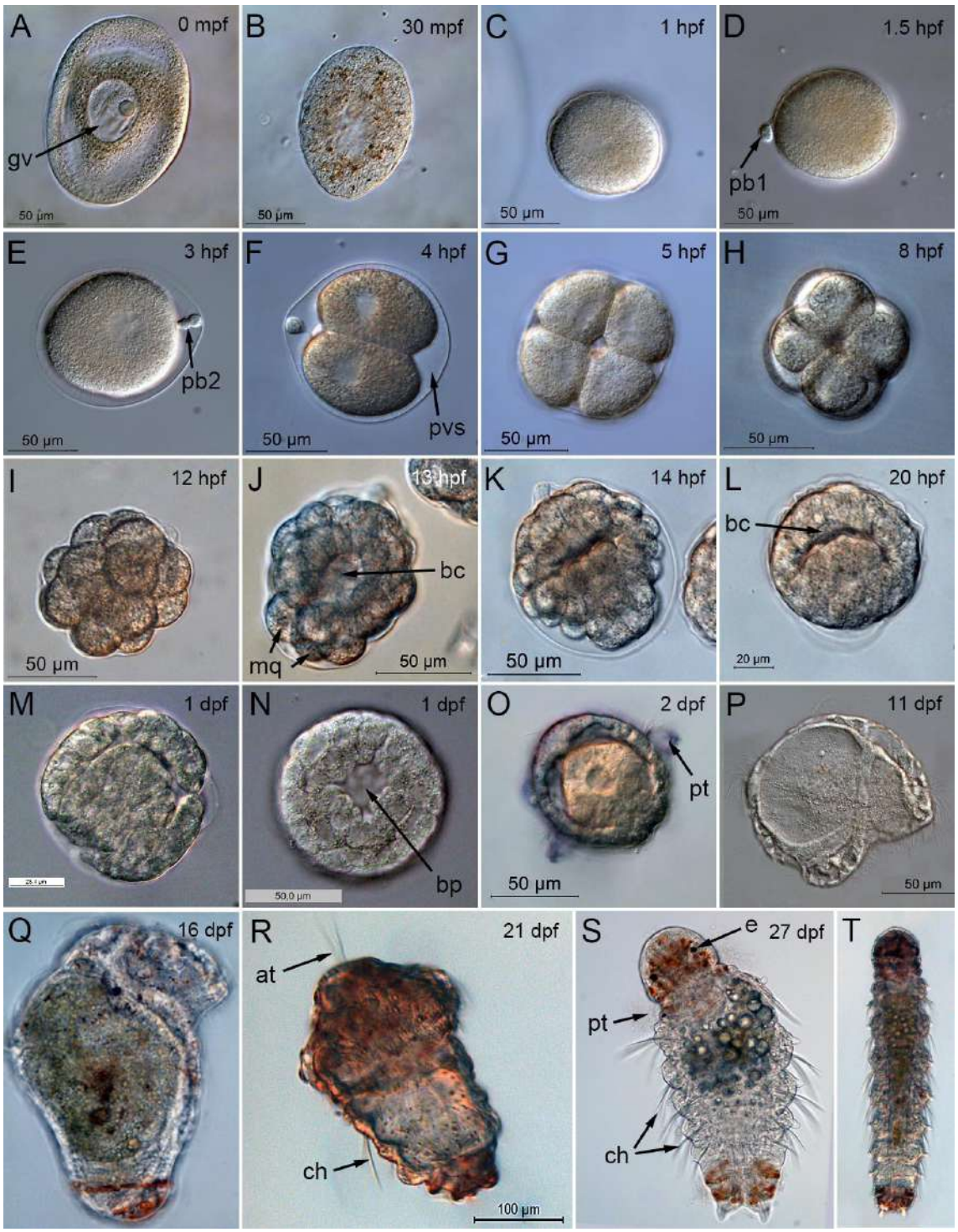

Figure 16. Development of *Ophelia limacina*.
A – oocyte, germinal vesicle (gv) is visible; **B** – ooplasmic segregation; **C** – segregation finished; **D** – the first polar body (pb1); **E** – the second polar body (pb2); **F** – two-cell stage, perivitelline space (pvs) is visible; **G** – 4-cell stage, all the blastomeres are equal; **H** – 8-cell stage, spiral pattern 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** – trochophore, cilia of prototroch (pt) are visible; **P** – feeding trochophore; **Q** – late metatrochophora, just before segments; **R** – nectochaeta, three segments, three pairs of chaetae (ch), apical tuft (at) are visible; **S** – settled nectochaeta, four segment worm, mo – mouth, e – eye, pt – prototroch, ch – chaetae. **T** – 3 month juvenile with 7 chaetigerous segments. dpf – days post fertilization.

Dinophilus taeniatus

Dinophillus taeniatus (Harmer, 1889) lives on filamentous algae inhabiting the lower tidal zone. They can be collected in the WSBS area at the Eremei 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. 17). 1-2 days later the female lays the egg clutches. Individual eggs can be isolated using dissecting needles.

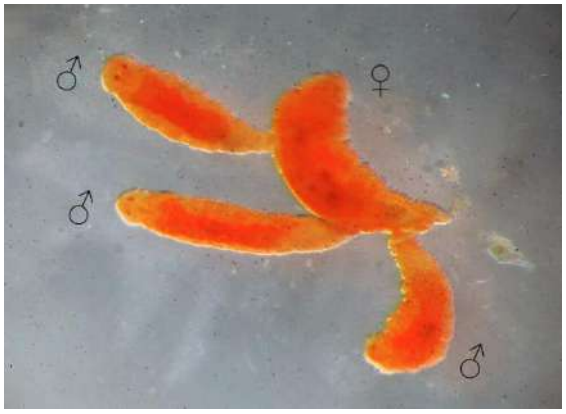

Figure 17. Copulation of *Dinophilus taeniatus*.
Three smaller males penetrates the single bigger female. © Tatiana Mayorova

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. 18 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. 18 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. 18 E, F). Blastopore closes on the ventral side. The juvenile worm then forms in the egg membranes after some time (Fig. 18 G).

Figure 18. Development of *Dinophilus taeniatus*.
A – zygote, pvs – perivitelline space, pb – polar body; **B** – 2-cell stage embryo, blastomere CD is bigger than AB; **C** – 4-cell stage, blastomer D is bigger than the others; **D** – 8-cell stage; **E** – blastula stage; **E** – post-gastrulation embryo; **G** – juvenile HRP-immunostained for cilia (brown), several ciliary belts (cb) are visible; **E** – sperm cells derived from male gonad. © Polina Bikmulina (A-F), Valeria Rousanova (G, H).

MOLLUSCA

POLYPLACOPHORA

The chiton *Tonicella marmorea* (Fabricius, 1780) is widely distributed on rocky substrates in the subtidal zone (Fig. 20 K). Its eggs are coral-red 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. 20 L). Eggs should be placed in a Petri dish with filtered seawater to observe typical spiral cleavage and further trochophore formation. Trochophores have a prototroch and an apical tuft (Fig. 20 M), 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.

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 *Epheria vincta* (Montagu, 1803) have the form of an open ring and are common on laminaria (Fig. 19 A). *Margarites groenlandicus* (Gmelin, 1791) lays eggs in mucous clumps (Fig. 19 B). Clutches of *Littorina obtusata* (Linnaeus, 1758) are tough and have an oval shape (Fig. 19 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. 19 F). *Neptunea despecta* (Linnaeus, 1758) and *Buccinum undatum* (Linnaeus, 1758) lay eggs in tight capsules, agglomerated in the form of a lump (Fig. 19 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. 19 I). 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. 190 J-L).

Testudinalia tessulata (Muller, 1776) habitats on the border of the intertidal and upper subtidal zones of the White Sea. Spawning can be induced by turning the mollusc upside down and warming the water to room temperature (Fig. 20 A). External fertilization and development occur in the water at the beginning of June. Spiral cleavage is an equal (Fig. 20 D-G). One day after the fertilization, an early trochophore is formed, which does not move, ciliary cells do not form prototroch. The trochophore about 30-39 hpf is started to swim (Fig. 20 B). It is with the apical cilia, the slit-like blastopore is almost closed, and on the dorsal side the shell gland is formed, which is still concave inward. 48-54 hpf, the velum develops, the telotroch shifts to the ventral side, there is a foot bud and a shell begins to form (Fig. 20 C). Veliger 90 hpf has a well-developed shell, which begins to calcify, foot, well-translucent liver through the shell and the rudiments of tentacles. Most likely the torsion occurs after 100 hpf, while the torsion veliger has a well-developed operculum, already a white shell and rather long head tentacles. By 170 hpf, the process of sedimentation begins, with the larvae floating-settling, eyes and well-developed tentacles, the test is completely calcified.

From time to time, the veliger of sea angel *Clione limacina* (Phipps, 1774) can be found in plankton that have a pronounced velyum and typical cup-shaped transparent shell (Fig. 20 I).

Figure 19. 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; **J** – *Nudibranchus rupium* egg clutches on the hydroid colonies; **K** – *Dendronotus frondosus* with its egg clutch; **L** – egg clutch of *Coryphella verrucosa*. © Alexander Semenov (H, J-L).

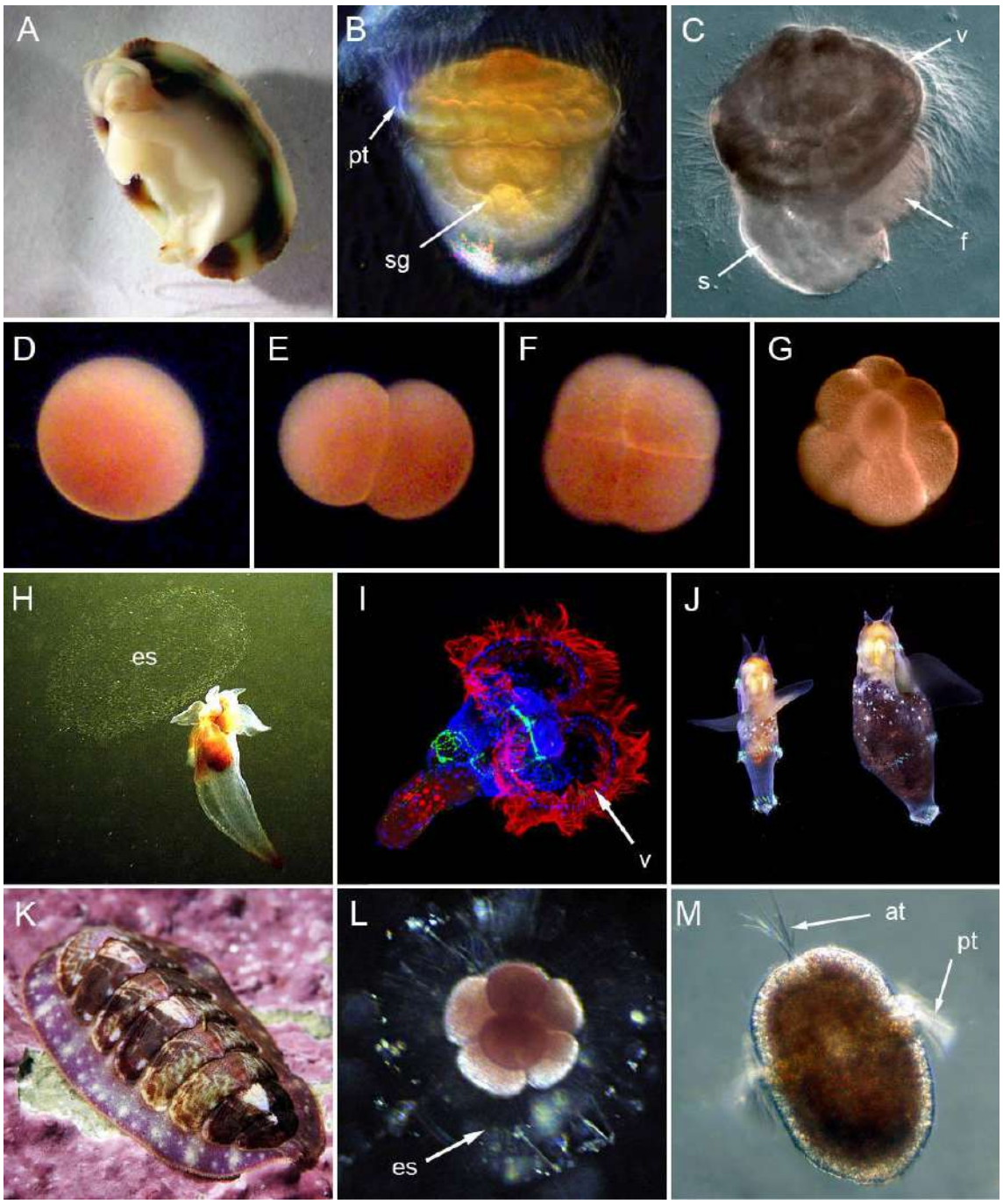

Figure 20. Development of White Sea molluscs.
A – *Testudinalia tessulata* inverted male releases the sperm; **B** –*T. tessulata* trochophore, pt – prototroch, sg – shell gland; **C** – *T. tessulata* veliger larva, v – velum, s – shell, f – foot; **D-G** – early development of *T. tessulata*; **D** – zygote; **E** – 2-cell embryo; **F** – 4-cell embryo; **G** – 8-cell embryo; **H** – *Clione limacina* with its pelagic egg clutch (ec); **I** – *C. limacina* trochophore fluorescent micrograph (nuclei are blue, cilia are red, serotonin neurons are green), v - velum; **J** – *C. limacina* polytrochal larvae; **K** – adult *Tonicella marmorea*; **L** – *T. marmorea* cleavage embryo in egg shell (es); **M** – *T. marmorea* trochophore, at – apical tuft, pt - prototroch. © Alexander Semenov (D, F, G).

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. 21). 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 21. ***Littorina saxatilis.***

L. saxatilis cleavage is total (holoblastic) and equal (Fig. 22 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. 22 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. 22 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. 22 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 macromers 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. 22 G), but velyum is weakly expressed and resorbed shortly before hatching of the juvenile snail (Fig. 22 H).

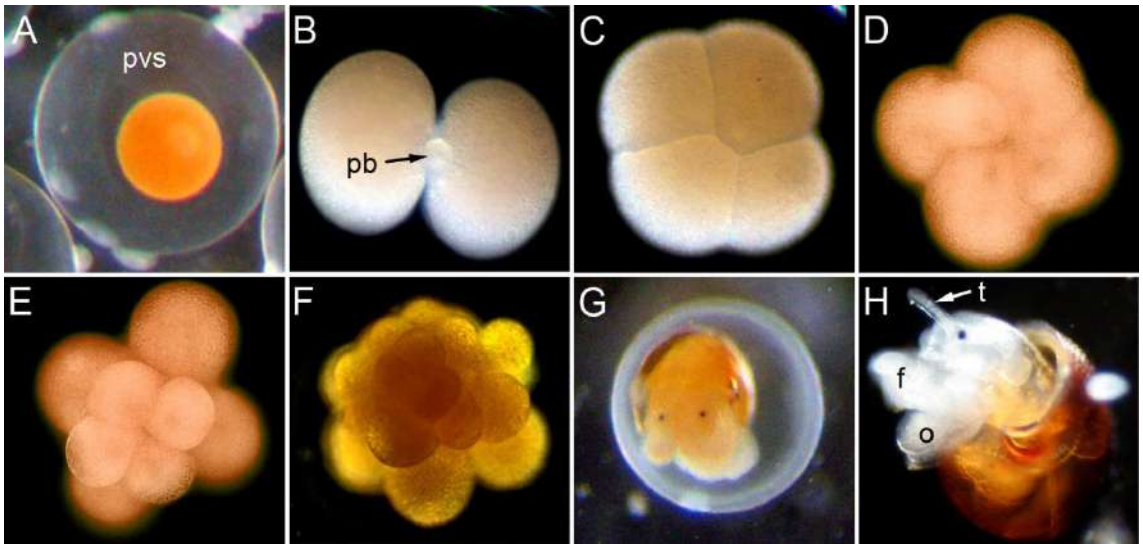

Figure 22. Development of *Littorina saxatilis*.
A – zygote, wide perivitelline space (pvs) is visible; **B** – 2-cell embryo, pb – polar body; **C** – 4-cell embryo, view from the vegetal pole, blastomeres D and B are in contact; **D** – the third cleavage division, view from the animal pole; **E** – 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.

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. 23). The eggs must be washed in several changes of filtered sea water and fertilized by adding a couple of sperm drops.

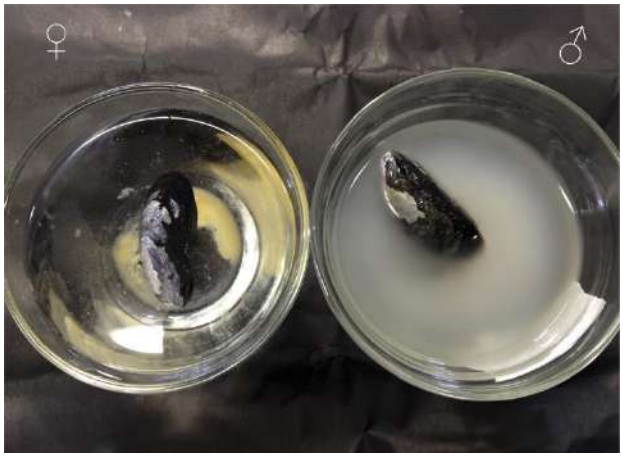

Figure 23. *Mytilus edulis* spawing after KCl injection.

Unfertilized eggs have an irregular shape (Fig. 24 A), but soon become spherical after fertilization; a perivitelline space appears and polar bodies are formed (Fig. 24 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. 24 C). Polar lobe material will become part of one of the blastomeres, which consequently will be larger (Fig. 24 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. 24 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. 24 H).

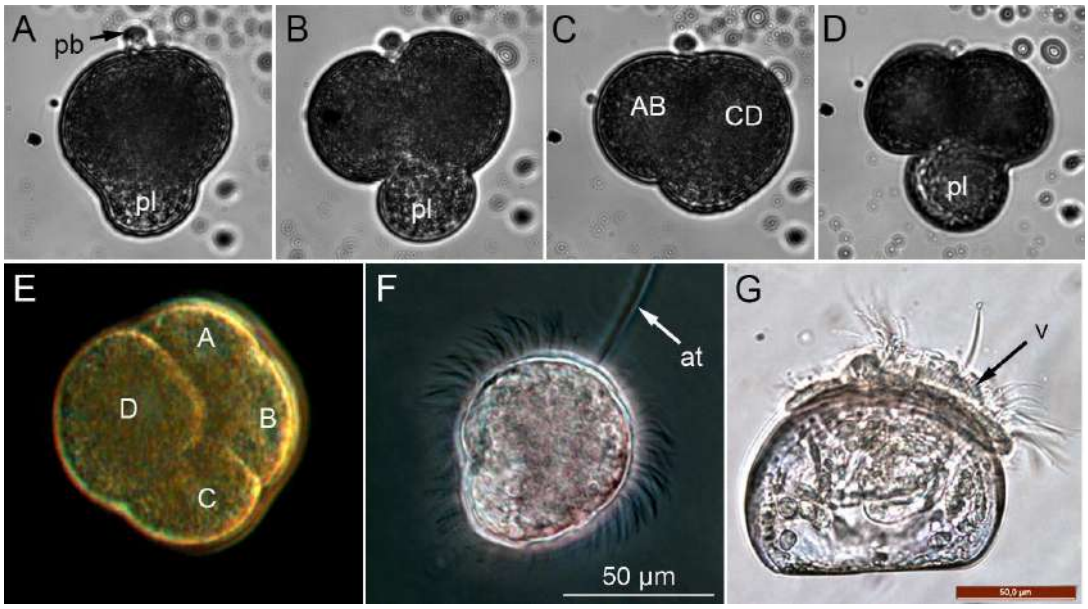

Figure 24. Development of *Mytilus edulis*.
A-D – timelapse videoregistration of cleavage divisions and polar lobe formation © Daniyal Saidov; **A** – zygote, the cytoplasm of polar lobe (pl) is visible at the vegetal pole, pb – polar body; **B** – the first cleavage division, pl – polar lobe; **C** – 2-cell embryo, blastomere CD is bigger than AB; **D** – the second cleavage division, polar lobe (pl) appeared again; **E** – 4-cell embryo, blastomer D is bigger than the others; **F** – ciliated larva (konchostoma), apical tuft (at) is on the anterior pole; **G** – veliger, v – velum, sh - shell.

NEMERTEA

Several species of nemerteans abound under rocks and seaweeds on silty substrates in the tidal zone. *Ramphogordius sanguineus* (Rathke, 1799) (Fig. 26 A) reproduces mainly asexually, by fragmentation. They are dioecious and fertilization is external. Planctonic pilidium larvae are formed then (Fig. 26 B, C). Pilidiums have a helmet shape with two lateral lobes and an apical plate with a cilia tuft (Fig. 26 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. 26 C). The adult worm body is formed from the imaginal discs during a catastrophic metamorphosis.

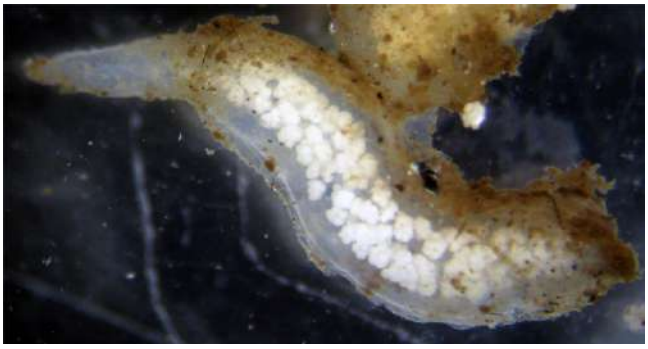

Figure 25. *Poseidon ruber* egg clutch.

Another widespread nemertean, *Poseidon ruber* (Muller, 1774), produces elongated mucous clutches containing 100-250 egg capsules (Fig. 25). Each egg capsule contains about 10 eggs, but only 1-2 of these develop (Fig. 26 D). The embryo forms a mouth (Fig. 26 E) that swallows all other eggs (Fig. 26 F). The fully formed worm leaves the egg shell then.

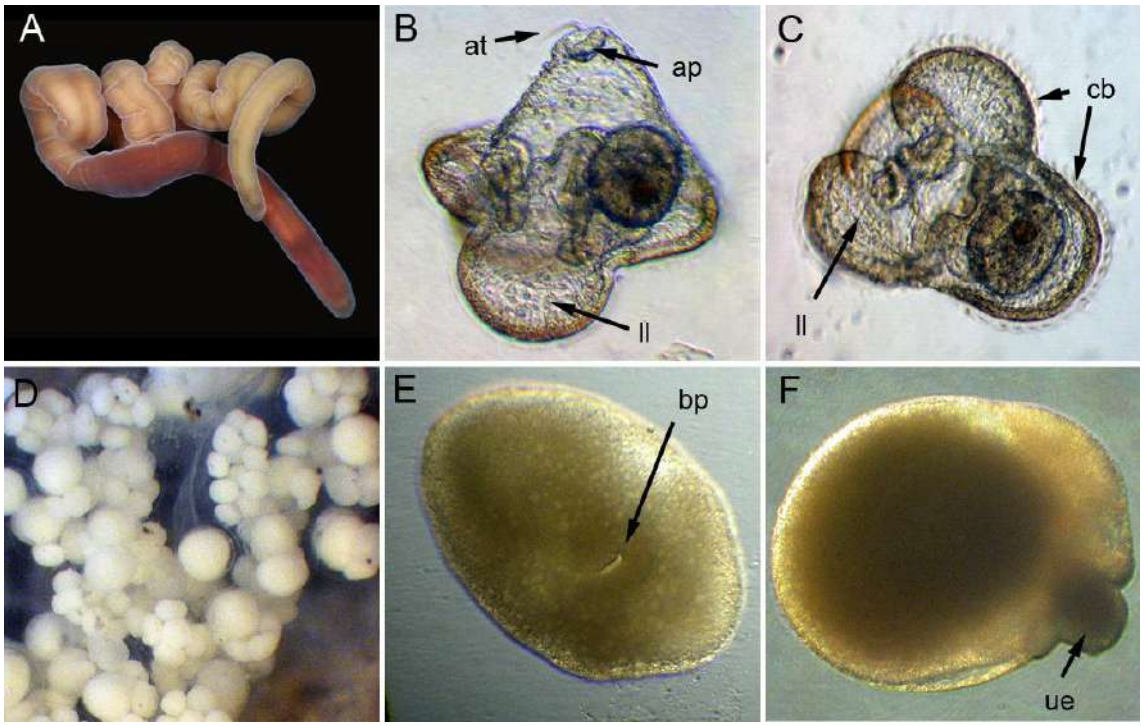

Figure 26. Development of White Sea nemerteans.
A – *Ramphogordius sanguineus* adult © www.aphotomarine.com; **B** – *R. sanguineus* pilidium larva, lateral view, ap – apical plate, at – apical tuft, ll – lateral lobe; **C** – *R. sanguineus* pilidium larva, lateral from lower surface, ll – lateral lobe, 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.

BRYOZOA

Colonies of *Cribrilina annulata* (Fabricius, 1780) are often presented on the red algae thalluses in the subtidal zone (Fig. 27 C). 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. 27 K). 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. 27 L, M). 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. 27 N) 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. 27 B). 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. 27 I). 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. 27 J). 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. 27 A). Complete uniform cleavage has a bilateral pattern (Fig. 27 D-F). The larvae are similar to cyphonaute (Fig. 27 H), but have an elongated shape, reduced gut and swim a very short time.

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. 27 O-R). Details of gastrulation and larval development remain unknown.

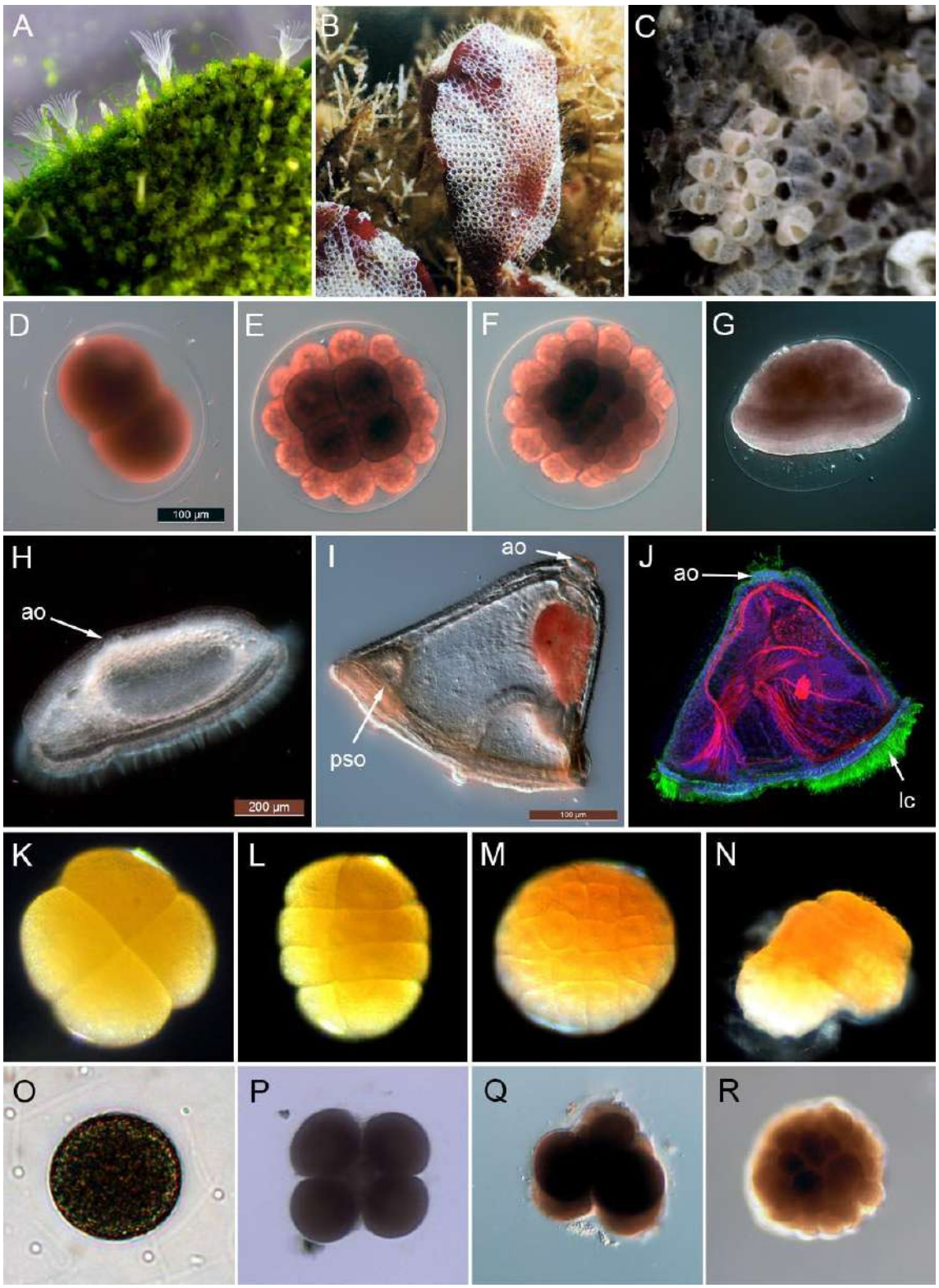

Figure 27. Development of White Sea bryozoans and brachiopods.
A – *Frustrellidra hispida* colony; **B** – *Electra pilosa* colony; **C** – *Cribrilina annulata* colony with embryos in the ovicells; **D-G** – embryonic development of *F. hispida* © Olga Kotenko; **D** – 2-cell embryo; **E** – 16-cell embryo; **F** – 32-cell embryo (placula); **G, H** – pseudo-cyphonaute larvae, ao – apical organ; **I** – *E. pilosa* cyphonaute larva, ao – apical organ, pso – pear-shaped organ; **J** – fluorescent micrograph of *E. pilosa* cyphonaute (nuclei are blue, cilia are green, muscles are red), ao – apical organ, lc – locomotor cilia; **K-N** – embryonic development of *C. annulata* © Natalia Budaeva; **K** – 4-cell embryo; **L, M** – cleavage; **N** – coronate larva; **O-R** – embryonic development of *Hemithiris psittacea* © Tatiana Kuzmina; **O** – zygote, sperms are visible around; **P** – 4-cell embryo; **Q** – the third cleavage division, blastomeres are unequal; **R** – morula-early blastula.

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. 28). 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. 29 A-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 29. *Pontonema vulgare* female.
Eggs (e) are visible inside the worm.

Polar bodies appear in about three hours after activation (Fig. 29 A, C). The first cleavage occurs 20 hours after activation (Fig. 29 C). Blastomeres have a constantly changing irregular shape in the intervals between divisions (Fig. 29 D, H). However, they take the form of a ball immediately prior to division (Fig. 29 C, E). Blastomeres have different configurations after the second cleavage (Fig. 29 E-H), but finally take on a rhombus pattern. Cleavage becomes asynchronous after the 16-cell stage. One of the blastomeres lags during divisions, deepens and forms the endoderm. The remaining blastomeres cover it and form a blastopore (Fig. 29 L). Later, the blastopore takes a slit-like shape (Fig. 29. M, N) 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).

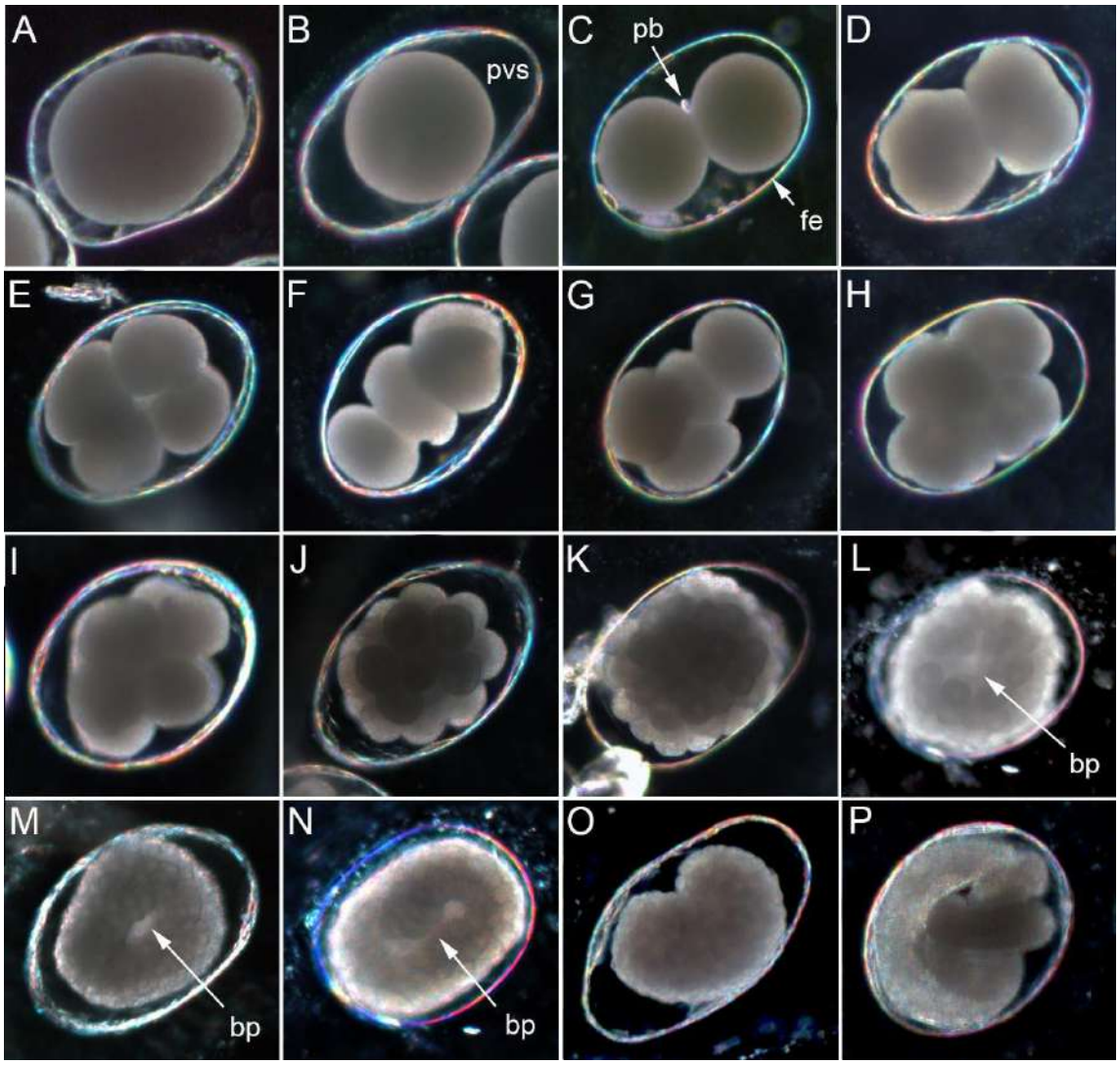

Figure 29. Development of *Pontonema vulgare*.
A – cortical reaction; **B** – zygote, fertilization envelope and perivitelline space (pvs) are visible; **C** – 2-cell embryo just after the first cleavage division, pb – polar bodies, fe – fertilization envelope; **D** – 2-cell embryo; **E-H** – 4-cell ebmryos that form tetrahedron, rhombus and T-shape patterns; **I** – 8-cell embryo; **J-K** – morula; **L** – gastrula with wide blastopore (bp); **M-N** – gastrula with slit-shaped blastopore (bp); **O** – post-gastrulation embryo; **P** – juvenile worm before hatching. © Valeria Rousanova.

ARTHROPODA

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. 30 F). Eggs are polylecital and development is direct (Fig. 30 L-M). Protonymphon larva has 3 pairs of appendages: appendages I of the corresponds to cheliphores of an adult animal, pairs II and III develop into palps and oviferous legs (Fig. 30 N).

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 (Fig. 30 A, B). Nauplii have one eye and three pairs of natatory cephalic appendages: uniramous antennules, biramous antennas and biramous mandibles. After moulting, the segmented thoracal 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. 30 B). They have a typical dorsal shield with tail needle and lateral horns. Following the metanauplius stage, a cyprid larva (Fig. 30 C) is formed that does not feed and which soon settles on a rocky substrate using antennulas, and metamorphoses.

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 (Fig. G-J).

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. 30 E, K).

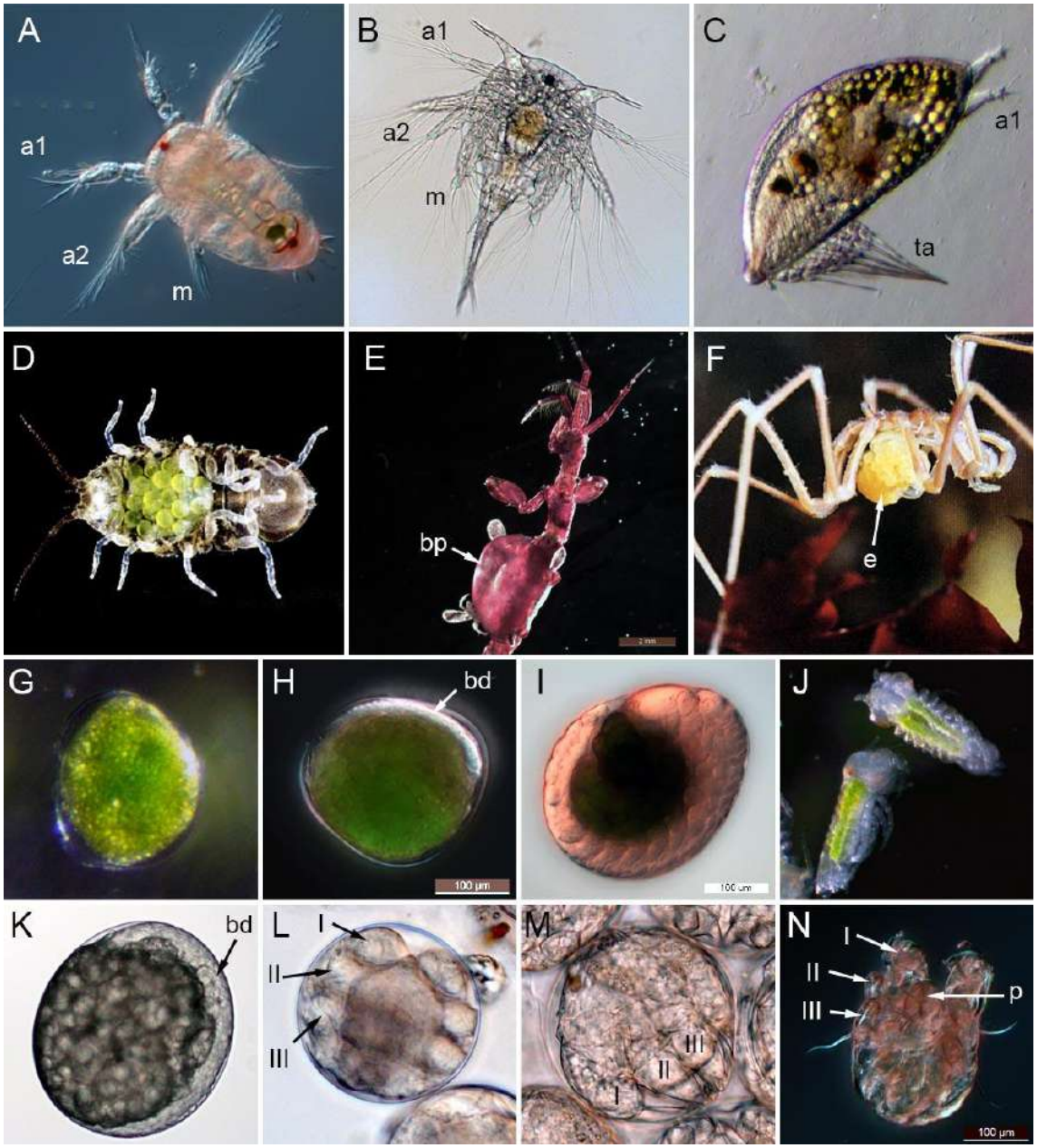

Figure 30. Development of White Sea arthropods.
A – *Pseudocalanus* nauplius, a1 – antennula, a2 – antenna, m – mandible; **B** – nauplius larva of cirripedian, a1 – antennula, a2 – antenna, m – mandible; **C** – cyprid larva, a1 – antennula with attachmet organ, ta – thoracic appentages; **D** – *Jaera albifrons* with green eggs; **E** – *Caprella septentrionalis*, bp – brood pouch; **F** – *Nymphon grossipes* male with the eggs (e) on the ovigerous legs; **G-J** – *Jaera albifrons* development; **G** – early cleavage; **H** – blastoderm (bd) formation; **I** – segmented embryo; **J** – hatched juvenile isopods; **K** – embryo of *C. septentrionalis* with blastoderm (bd); **L-N** – *N. grossipes* development, I-III – appentages; **N** – hatched protonymphon larva, p – proboscis. © Alexander Semenov (D, F).

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 (Fig. 31 A, B). Amphipods copulate and are held together for a long time (Fig. 31 C). 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.

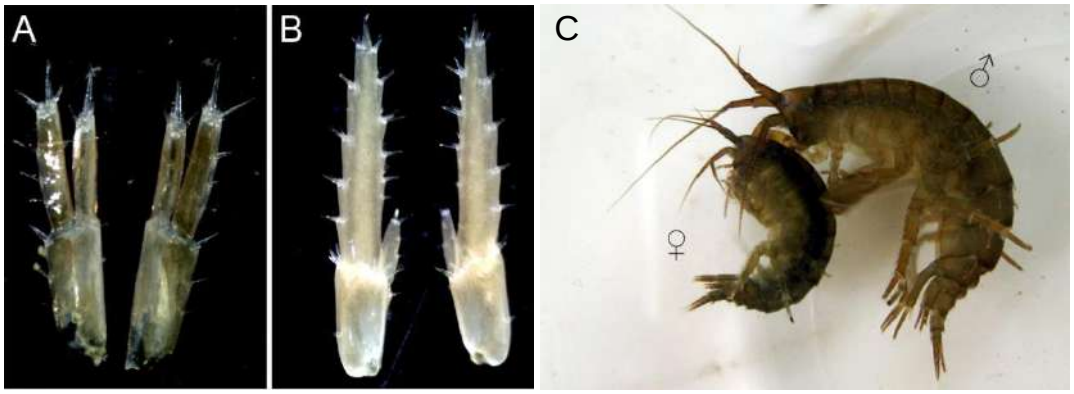

Figure 31. The White Sea gammarides.
A – uropods of *Gammarus duebeni*; **B** – uropods of *Marinogammarus obtusatus*; **C** – mating of *M. obtusatus*.

The eggs of amphipods contain significant amounts of yolk (polylecithal) that is orange-red (Fig. 32 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. 32 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. 32 D). Blastoderm formation begins about 36 hours after fertilization. Nuclei, surrounded by whitish cytoplasm, emerge on the blastomeres' surface (Fig. 32 E) and are subsequently separated from the yolk mass. These surface cells form the blastoderm (Fig. 32 F), continue to divide and gradually surround the yolk mass. Germinal disc formation, gastrulation and morphogenesis occur in the later stages of development (Fig. 32 G, H).

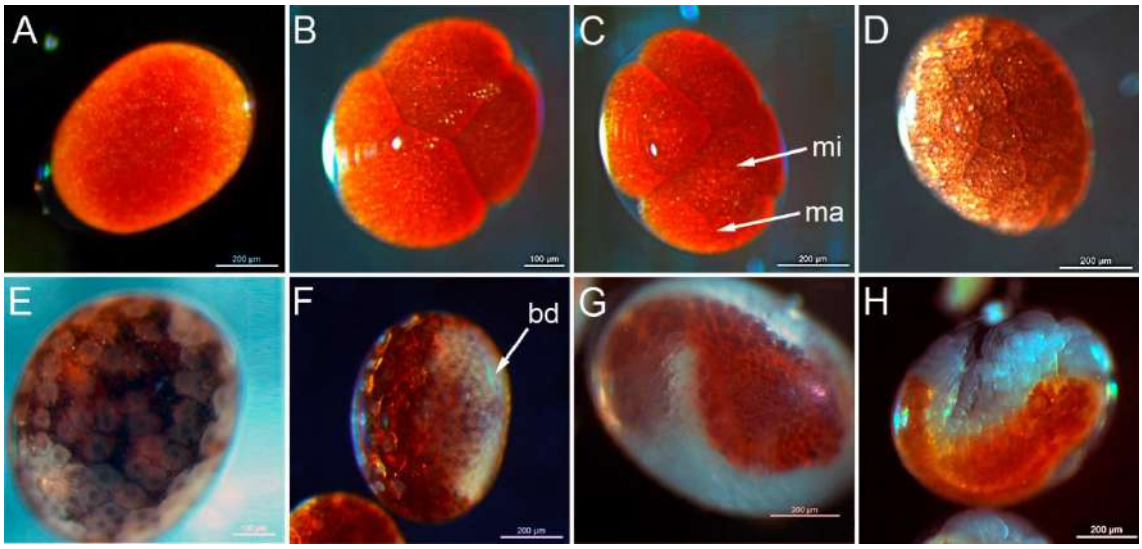

Figure 32. Development of *Marinogammarus obtusatus*.
A – zygote; **B** – 4-cell stage; **C** – 8-cell stage, ma – macromeres, mi – micromeres; **D** – morula; **E-F** – blastoderm (bd) formation; **G-H** – segmented late embryos.

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 gonopors 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. 33 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. 33 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.

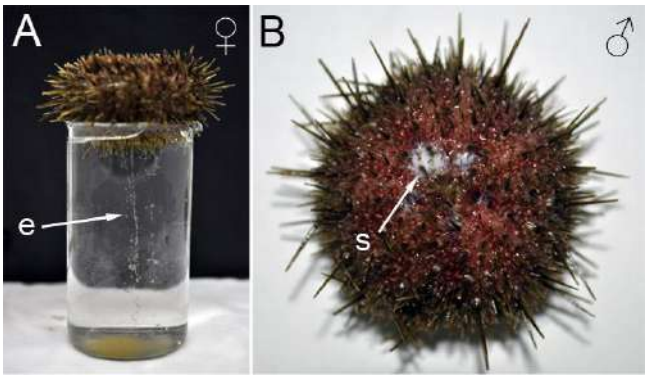

Figure 33. Obtaining of the gametes of sea urchin *Strongylocentrotus pallidus* by KCl injection.
A – spawning female, e – eggs; **B** – spawning male, s – sperm.

A fertilization envelope occurs within 5-10 minutes following fertilization (Fig 34 A). Cleavage is holoblastic and radial (Fig. 34 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 macromers and four micromeres lying on the vegetal pole (Fig. 34 D). The unevenness of blastomeres disappears by the blastula stage (Fig. 34 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. 34 F) and hatching of mesenchyme blastula occurs after seven days (Fig. 34 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. 34 K) and coelomic pouches are formed later by separating from the top of archenteron (Fig. 34 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. 34 M). Ehinopluteus larvae are formed at a later stage (Fig. 34 N-P). Each of the four arms of the early pluteus has a skeletal spicule inside (Fig. 34 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.

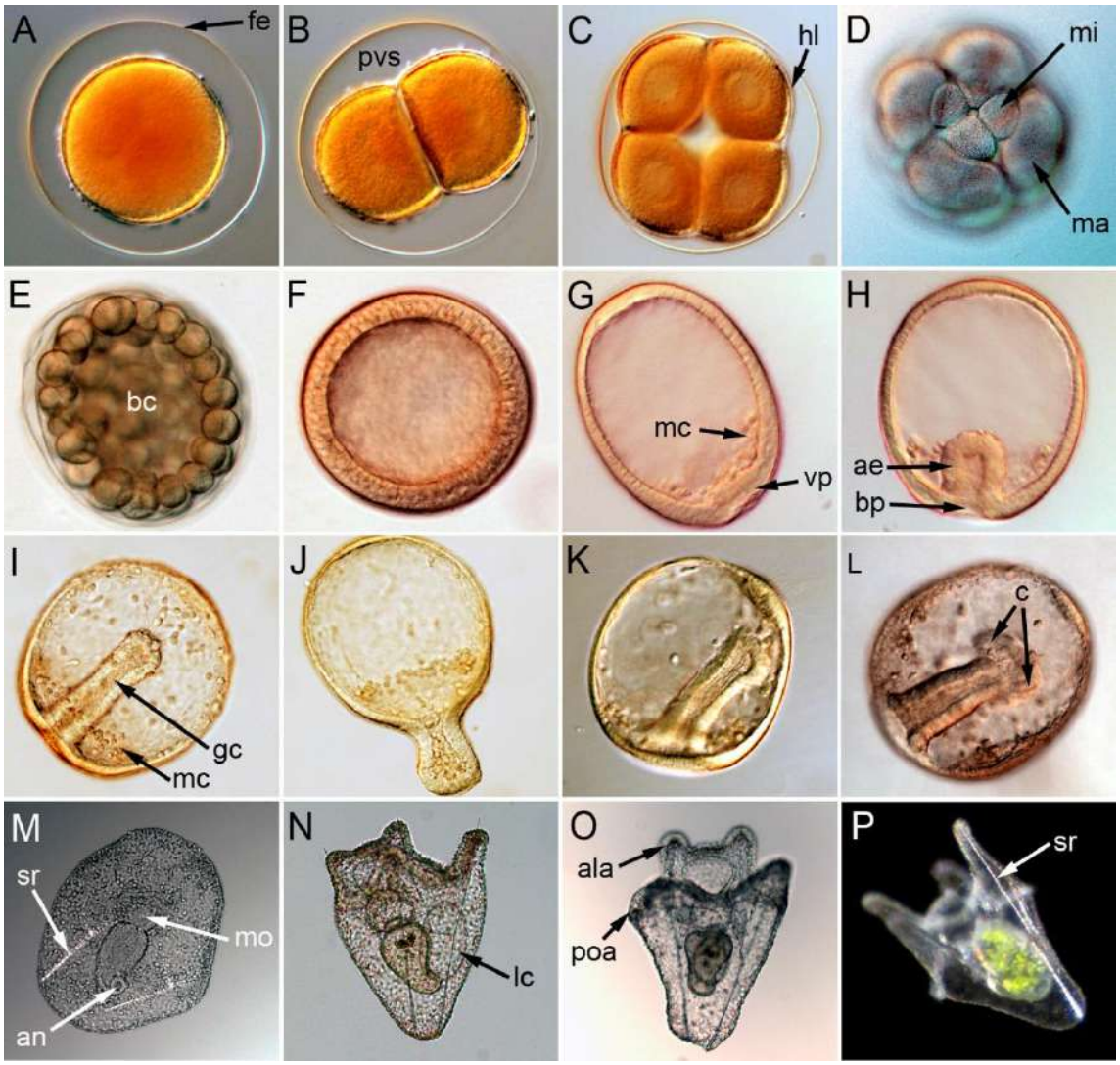

Figure 34. Development of *Strongylocentrotus pallidus*.
A – zygote, fertilization envelop (fe) is visible; **B** – 2-cell stage, pvs – perivitelline space; **C** – 4-cell stage, hyaline layer (hl) is visible; **D** – 16-cell embryo, view from vegetal pole, mi – micromeres, ma – macromeres; **E** – early 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, 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.

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. 38 A-C), where optimal conditions are provided by aeration. Formed juvenile starfishes leave the brood pouch through breaks in the membrane. Eggs and lecitotrophic larvae of *Crossaster papposus* (Linnaeus, 1768) (Fig. 38 E) are found in plankton during March.

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. 35 A, B), while testes are whitish (Fig. 35 D, E). An ovary should be cut, wrapped in a piece of fine mesh (about 200 µm) (Fig. 35 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. 36 A), while the cytoplasm of a mature egg is homogeneous (Fig. 36 B). Testes should be placed in a small amount of filtered seawater (Fig. 35 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.

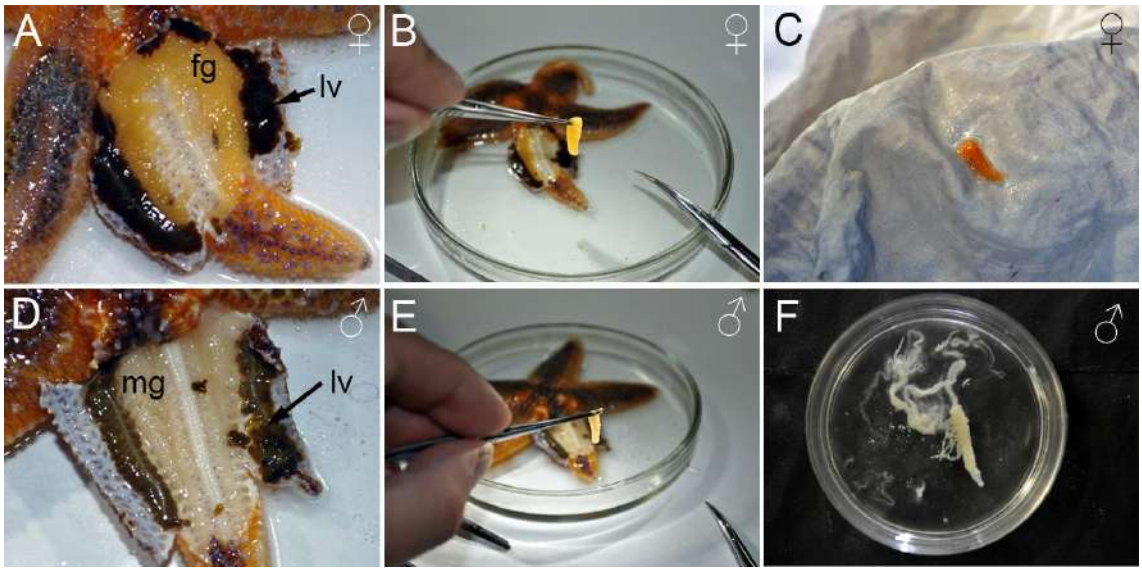

Figure 35. *Asterias rubens* gametes obtaining.
A – dissected arm of female, fg – female gonad, lv – liver; **B** – orange ovary isolated from female; **C** – ovary placed on a piece of mesh for washing out oocytes; **D** – dissected arm of male, mg – male gonad, lv – liver; **E** – whitish testis isolated from male; **F** – sperm released from the testis.

A fertilization envelope and polar bodies will appear soon after fertilization (Fig. 36 C, D). Cleavage of starfish is holoblastic; the first two cleavage furrows are meridional and divide the zygote into four equal blastomeres (Fig. 36 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. 36 G). The epithelial cilia-covered blastula is formed once 20 hours have passed after fertilization (Fig. 36 H). A vegetative plate appears and primary mesenchyme cells begin to immigrate to blastocoel by the time of hatching (Fig. 36 I).

Gastrulation occurs about 30-40 hours after fertilization. A tubular archenteron forms as a result of invagination of the blastula vegetative wall (Fig. 36 J). During gastrulation, the archenteron is lengthened and deviates to the ventral wall of the larva (Fig. 36 K). A pair of coelomic pouches is separated from the archenteron at later stages of development (Fig. 36 L). Early bilaterally

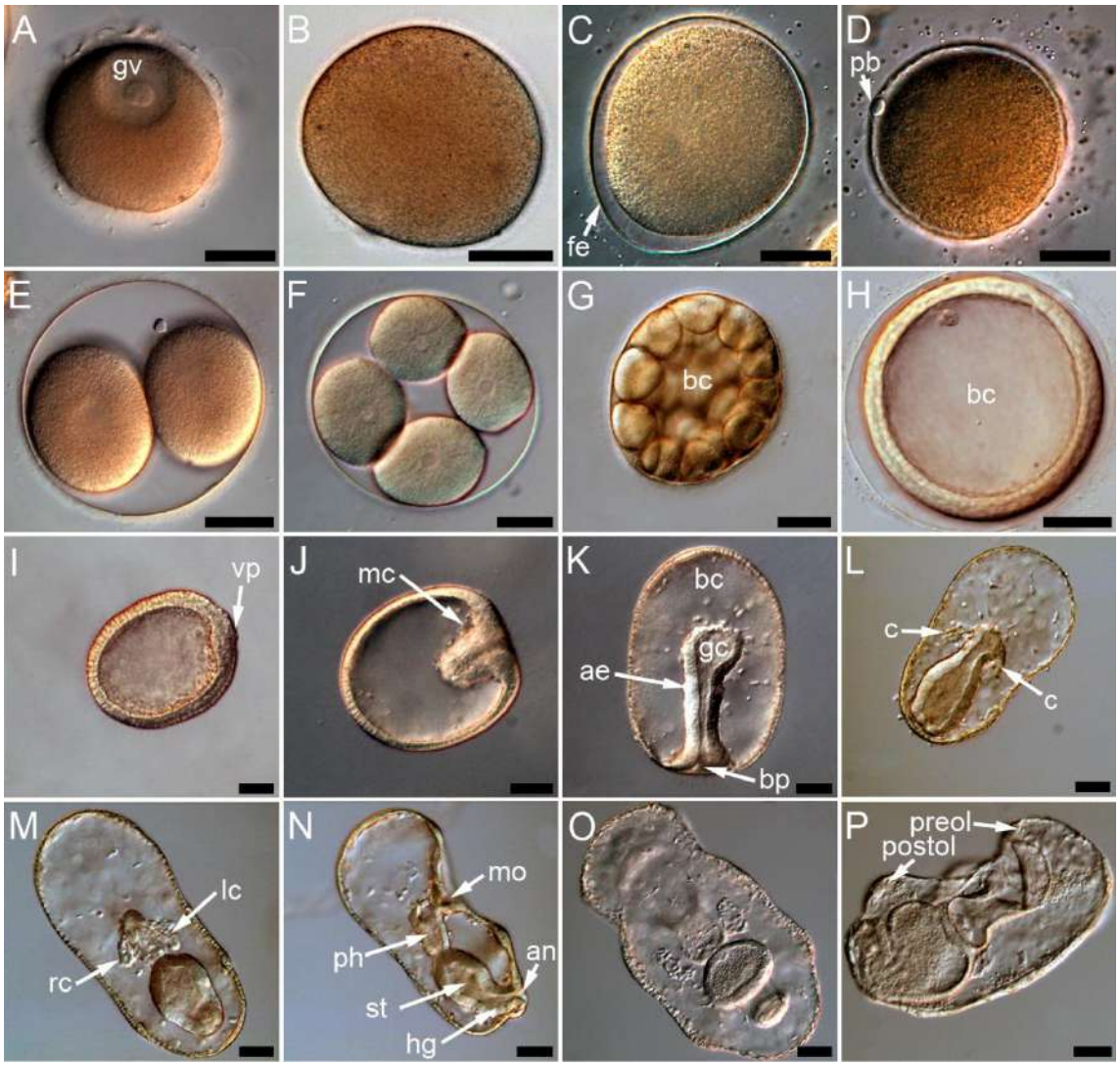

Figure 36. Development of *Asterias rubens*.
A – immature oocyte with germinal vesicle (gv); **B** – mature egg; **C** – fertilized egg with fertilization envelope (fe); **D** – zygote with polar body (pb); **E** – 2-cell embryo; **F** – 4-cell embryo; **G** – early blastula, bc – blastocoel; **H** – 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 breaked 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.

symmetrical larva called dipleurula forms (Fig. 36 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. 36 N).

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. 37 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. 36 N, P; 37 B). The preoral lobe is formed anteriorly of the mouth, while the anus is on the postoral lobe (Fig. 36 P; 37 A, B). Ciliated bands appear at the edges of the blades and expand as outgrowths, called arms (Fig. 37 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. 37 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. 37 F). A juvenile starfish arises from this area of the larva during the subsequent metamorphosis.

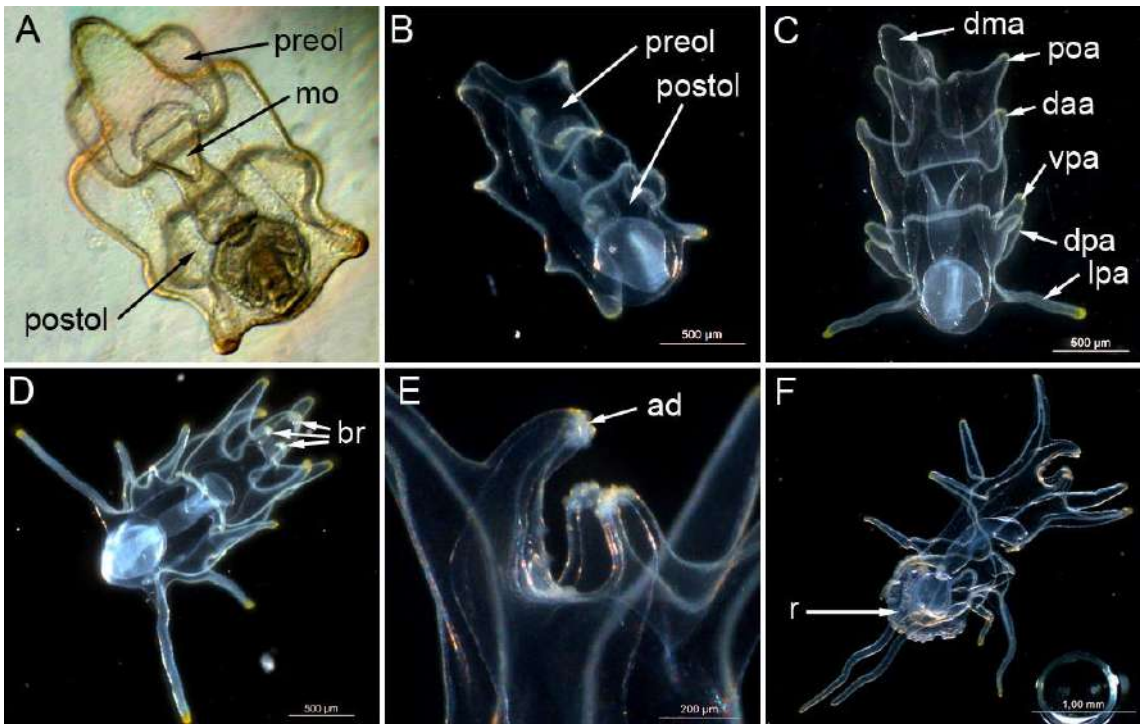

Figure 37. Larvae and metamorphosis of *Asterias rubens*.
A-B – bippinaria, preol – preoral lobe, postol – postoral lobe, mo – mouth; **C-D** – brachiolaria with 11 larval arms: unpaired dorso median one (dma), paired preoral (poa), paired dorsoanterior (daa), paired ventroposterior (vpa), paired dorsoposterior (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).

OPHIUROIDEA

Brittle stars *Ophiopholis aculeata* (Linnaeus, 1767) and *Ophiura robusta* (Ayres, 1851) spawn eggs and sperm during June-July (Fig. 38 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. 38 H). During development, the postoral arms of ophiopluteus become extremely long and rudiment is formed; these become a brittle star body after metamorphosis (Fig. 38 I).

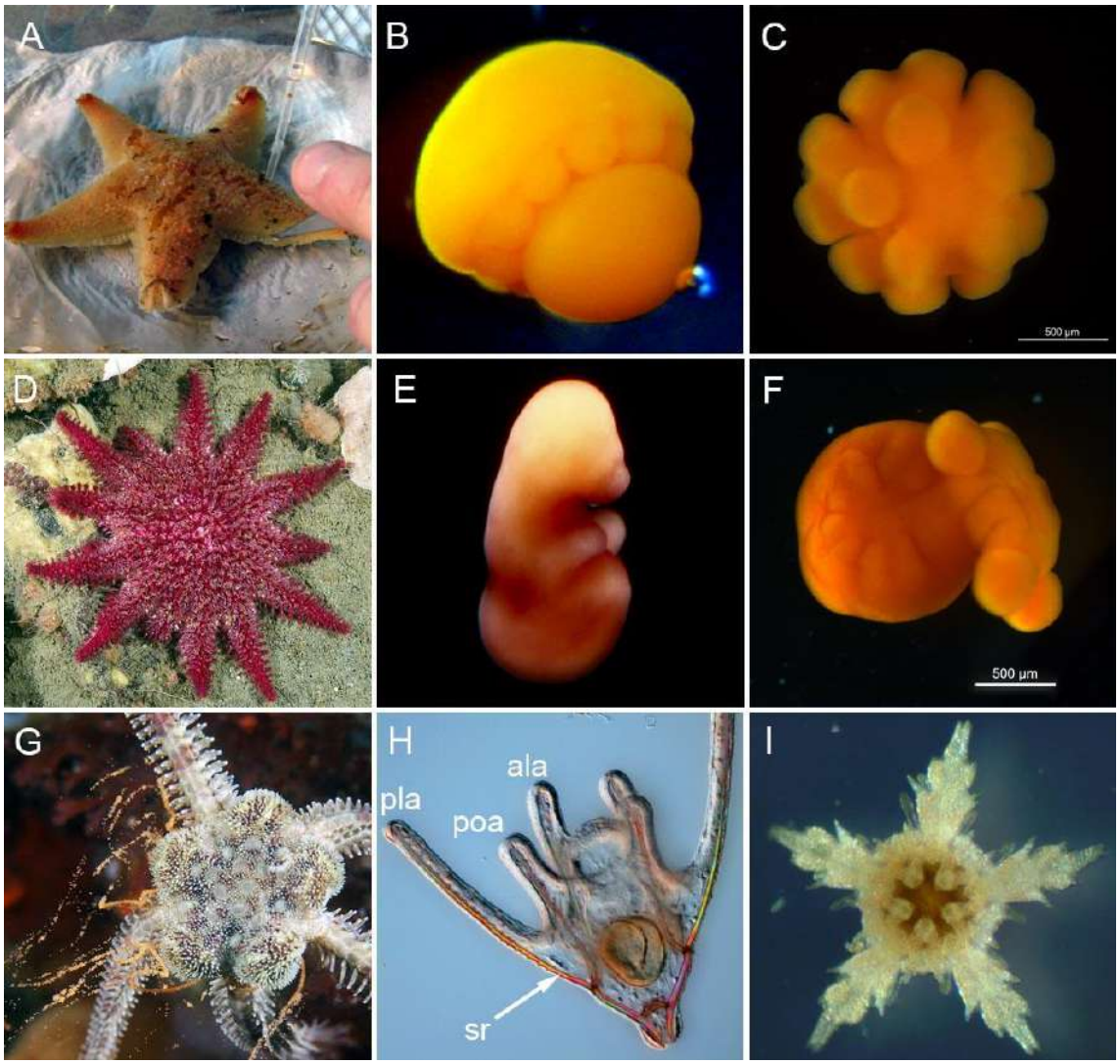

Figure 38. Development of White Sea echinoderms.
A – *Pteraster militaris* embryos obtaining from the subdorsal cavity of the female; **B-C** – *P. militaris* lecitotrophic larvae (mesogens) © Natalia Budaeva; **D** – *Crossaster papposus* adult; **E** – *C. papposus* lecitotrophic larva; **F** – *Henricia* sp. lecitotrophic larva; **G** – spawing *Ophiopholis aculeata* female; **H** – ophiopluteus, skeletal rods (sr) are visible, ala – anteriolateral arm, poa – postoral arm, pla – posterolateral arm; **I** – juvenile brittle star.

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. 39 A), *Molgula griffithsii* (MacLeay, 1825) (Fig. 39 B). 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 (Fig. 39), gastrulatuon by invagination and the formation of "tadpole" larva can be observed. The larva is folded inside the egg membrane prior to hatching. 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.

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.

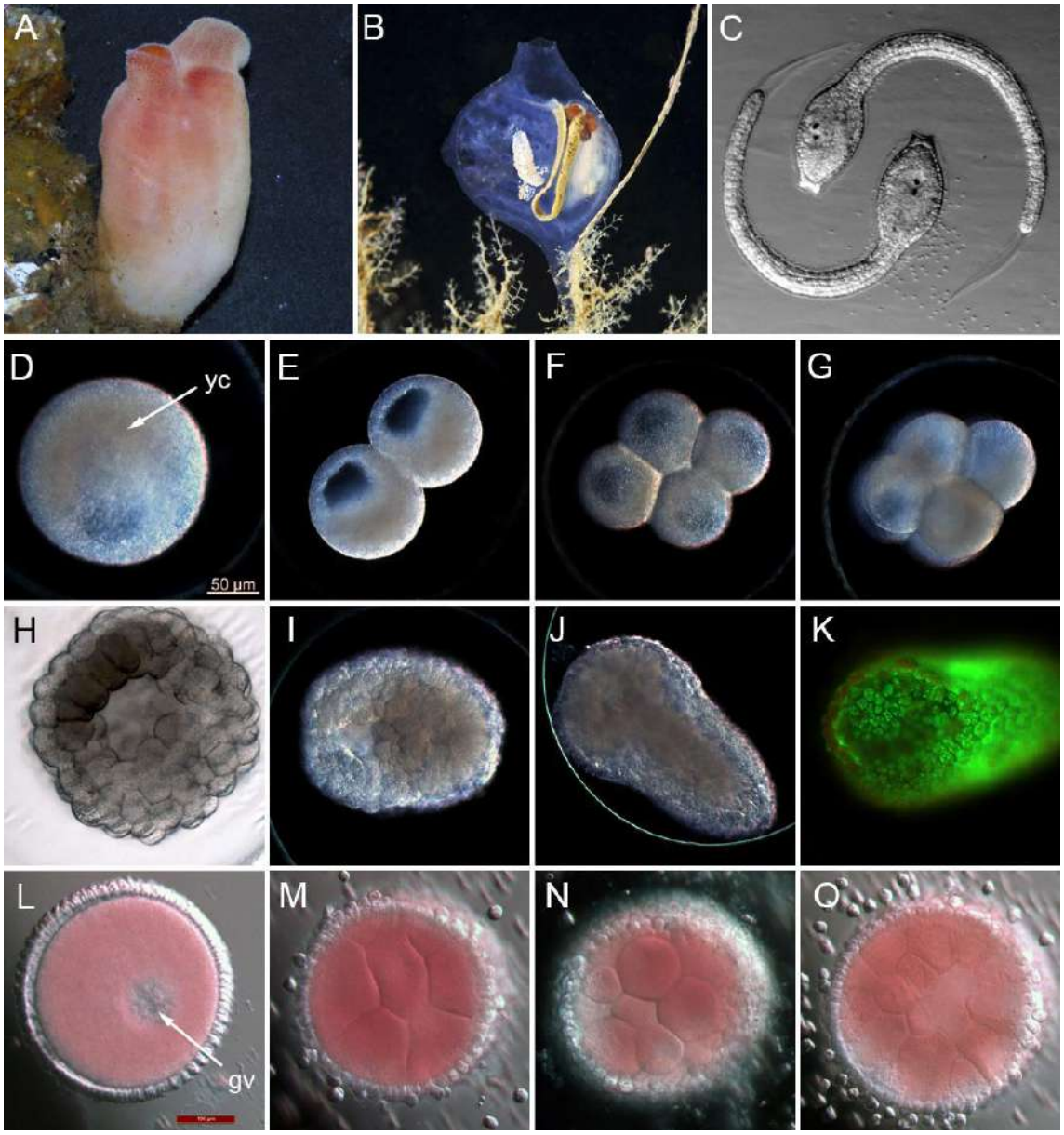

Figure 39. Development of White Sea ascidians.
A – adult *Halocynthia pyriformis*; **B** – adult *Molgula griffithsii* © Alexander Semenov; **C** – *H. pyriformis* larvae; **D-K** – development of *H. pyriformis*; **D** – oocyte, yellow crescent (yc) is visible; **E** – 2-cell embryo; **F** – 4-cell embryo; **G** – 8-cell embryo; **H** – gastrula; **I** – late gastrula; **J** – neurula; **K** – fluorescent micrograph of neurula stained vitally by Acridin Orange, yolk granules are green; **L-O** – development of *M. griffithsii*; **L** – oocyte, germinal vesicle (gv) is visible; **M-Q** – cleavage stage embryos © Renata Yalchina.

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Более 30 лет Диаэм оснащает лаборатории, и потому обладает уникальным опытом в своей области. Мы поставляем оборудование, расходные материалы и химические реактивы российских и зарубежных производителей для нужд научных биологических, химических, медицинских лабораторий, контрольных и санитарных лабораторий и т.п. В области эмбриологии компания **Диаэм** предлагает оборудование и расходные материалы для разных методов исследования: гистологии и иммуногистохимии, кариотипирования и FISH-анализа, вестерн-блоттинга, масс-спектрометрии, изучения экспрессии на уровне единичных клеток, секвенирования. Мы предлагаем микроскопы (светлопольные, флуоресцентные, конфокальные, электронные), микроманипуляторы и системы лазерной микродиссекции, холодильное и морозильное оборудование и многое другое. Компания **Диаэм** напрямую сотрудничает с такими фирмами, как Abcam, Thermo Fisher, Merck, Bio-Rad и многими другими, обеспечивая быструю поставку и невысокие цены.

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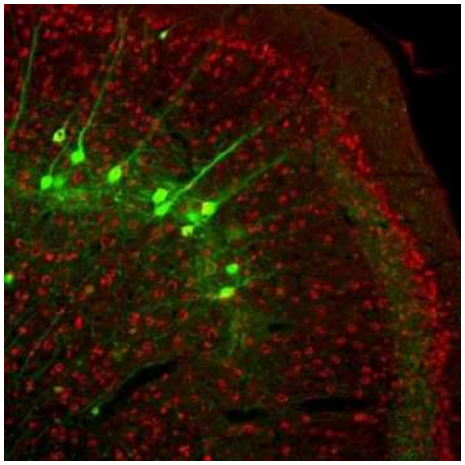

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