



Summer course in embryology of marine invertebrates-2

The White Sea Station, 2016

TENTATIVE PROGRAMME

Daily schedule: from 9:00 up to 20:00 - sampling and lab work, from 21:00 to 22:00 evening lectures. The labs are always open and people can work there as long as they want. Normally we have breakfast from 8:00 to 9:00, lunch from 13:00 to 14:00, and dinner 20:00–21:00. Time of 15-30 minutes coffee breaks is flexible, usually between lunch and dinner (except arrival and departure days). The correct time of field trips will be determined by the tidal schedule.

As needed on-duty students (by pairs) are responsible for the plankton sampling at the pier of the station or with row boats.

DAY 1 - SUNDAY - 12.06.2016

Arrival and accommodation at the White Sea Biological Station (WSBS); organization of the workplaces in the laboratory.

Lunch (14.00 – 15.00)

Excursion around the WSBS (15.00-17.00): **Alexander Tzetlin and Yulia Khramova**

Laboratory introduction (17.00-17.30): **Nadezhda Rimskaya-Korsakova** - «**The basic principles of working with cultures of embryos and larvae in a laboratory; review accessible equipment and literature; general principles of zoological drawing**»

Field excursion (17:30-18:30): **Yulia Kraus and Igor Kosevich** - plankton sampling with row boats and sorting of plankton samples in the laboratory; it is planned to obtain the gametes of hydromedusae next day; focus species - *Aglantha digitale*, *Bougainvillia superciliaris* *Sarsia tubulosa*.

Welcome lecture (19.00-20.00): **Alexander Tzetlin** - «**History of the WSBS: past, present and future**»

Dinner (20.00-21.00)

Welcome party (21.00-23.00): introduction of course participants (student and instructors), we encourage our students to give short talks or poster presentations devoted to their scientific interest.

DAY 2 - MONDAY - 13.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.00): **Nadezhda Rimskaya-Korsakova** - «**Introduction to embryology of marine invertebrates: the types of eggs, the types of cleavage, blastula, gastrulation, the formation of the mesoderm; organogenesis; metamorphoses**»

Practical session (10.00-13:00): **Yulia Kraus and Igor Kosevich** - sorting of fresh plankton samples, observation of the various hydrozoan and young scyphozoan jellyfishes and comb jellies. Collecting fertilized eggs and embryos of *Aglantha digitale*, *Bougainvillia superciliaris* and *Sarsia tubulosa* that were placed into the lab the day before; observation and drawing of early development of hydrozoan cnidarians.

Training skill: **Yulia Kraus and Igor Kosevich** - identifying proliferating cells by EdU labeling. Labelling and fixation of *A. digitale* embryos and larvae.

Lunch (13.00 – 14.00)

Lecture (14.00 – 15.00) : **Leica specialists** - “**Leica Microsystems: equipment and technologies for embryology**”

Practical session (15:00-18:30, parallel activities): **Leica specialists** work individually with each pair of students - **introduction to light microscopy for visualization and documentation of embryos of different types** (using cnidarian embryos and larvae).

Yulia Kraus and Igor Kosevich – the rest of the group works on observation and drawing of hydrozoan planula – larvae (*A. digitale*, *B. superciliaris*) and direct transformation of planulae into medusae (metamorphosis of *A. digitale*) .

Field excursion (18.30-20.00): collection of the various hydropolyps (especially, *Gonothyrea loveni*), various ephyrae, scyphistomae and anthozoan *Aulactinia stella* at the Eremai Rapids. Besides, collection of the egg clutches of polychaetes (Annelida) and nudibranchs (Mollusca). Sorting of samples in the laboratory.

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Mark Martindale - «Reproduction and Development of Cnidaria and Ctenophora»**

DAY 3 - TUESDAY - 14.06.2016

Breakfast (8.00-9.00)

Lecture (9:00-10:00): **Yulia Kraus - «Diversity and plasticity of cnidarian developmental pathways»**

Practical session (10.30-13:00): observation and drawing of metamorphosis of the *A. digitale*; young scyphozoan medusae and developing actinia polyps isolated from the maternal gastric cavity.

Training skill (10:30-13:00): **Yulia Kraus and Igor Kosevich** - identifying proliferating cells by EdU labeling (start of the protocol before morning lecture). Washing, click-reaction, glycerol embedding etc.

Lunch (13.00 – 14.00)

Lecture (14.00 – 15.00) : **Olympus specialists - “Olympus: equipment and technologies for embryology”**

Practical session (15:00-18:30, parallel activities): **Olympus specialists** work individually with each pair of students - **introduction to light microscopy for visualization and documentation of embryos of different types** (using cnidarian embryos and larvae).

Lev Belousov and Denis Nikishin - development and metamorphosis of *Gonothyraea loveni*, a hydropolyp with the reduced medusa form.

Field excursion (18.30-20.00): collection of various annelids and clutches of *Phyllodoce maculata* inhabiting intertidal zone in close vicinity to the station; sorting animals and clutches in the laboratory

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Lev Belousov - "Thecate hydroids (Leptomedusae) as morphogenetic machines».**

DAY 4 - Wednesday- 15.06.2016

Breakfast (8.00-9.00)

Lecture (9:00-10:00): **Andreas Hejnl - «Introduction to Spiralia»**

Practical session (10.00-13:00): **Natalia Budaeva and Andreas Hejnl - setting of fertilization, observation of ooplasmic segregation and stages of early development of annelid *Ophelia limacina*. Copulation of annelid *Dinophilus gyrociliatus*.**

Lunch (13.00 – 14.00)

Lecture (14.00 – 15.00): **Natalia Budaeva - «Reproduction and Development of Annelida»**

Practical session (15.00-18.00, parallel activities): **Natalia Budaeva and Andreas Hejnl** - observation and drawing of diversity of trochophore larvae from the plankton

Yulia Kraus and Igor Kosevich – cLSM sessions for visualization of the proliferating embryonic and larval cells.

Practical session (18.00-20.00): continuation of metamorphosis experiments of *Gonothyrea loveni*, working with hydroid and annelid cultures.

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Yulia Kraus - «What do not we know about the embryonic organizers?»**

DAY 5 - Thursday - 16.06.2016

Breakfast (8.00-9.00)

Practical session (9.00-11.00, parallel activities): **Natalia Budaeva and Nadezhda Rimskaya-Korsakova** - development of annelid *Phyllodoce maculata* laying egg clutches and viviparous *Circeis armoricana*.

Andreas Hejnl - immunocytochemistry and confocal microscopy - Fixation and Blocking of specimens.

Lunch (13.00 – 14.00)

Lecture (14.00-15.30): **Andreas Hejnl and Andreas Wanninger - introduction to immunocytochemistry and confocal microscopy**

Practical session (15.30-18.30 parallel activities):

1) students projects on immunocytochemistry and confocal microscopy using various larvae from the plankton samples (students will work in pairs; 1 instructor per student pair: **Andreas Hejnl, Andreas Wanninger, Denis Nikishin, Stanislav Kremnyov, Nadezhda Rimskaya-Korsakova**). Continuation of projects: primary antibodies application

2) **Natalia Budaeva and Nadezhda Rimskaya-Korsakova** - continuation working with earlier set annelid cultures.

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Vladimir Malakhov - «Ciliary bands and filtration mechanisms in larvae and adults of invertebrates»**

DAY 6 - Friday - 17.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.00): **Andreas Wanninger - «Development of Gastropoda (Mollusca)»**

Practical session (10.00-13.00): **Andreas Wanninger and Yulia Khramova**- gastropod development: *Littorina saxatilis* and limpet *Testudinalia tessellata*. Dissection of the viviparous gastropod *Littorina saxatilis*, observation of the different cleavage stages. Culture set-up of the collected cleavage stages. Stimulation of spawning of limpets *Testudinalia tessellata*, fertilization, cultures set-up.

Lunch (13.00 – 14.00)

Practical session (14.00-20.00, parallel activities):

1) continuation of projects on immunocytochemistry: secondary antibodies application.

2) sorting plankton sampling and drawing the molluscan embryos and larvae (chitons, gastropods, bivalves). Observation and drawing of various molluscan egg clutches collected earlier at the Eremai rapids (like *Epheria vincta*, *Buccinum undatum*, *Margarites helycinus*, *Littorina obtusata*, *Cryptonatica affinis*, *Dendronotus frondosus* and various nudibranchs).

3) continuation working with earlier set-up cultures.

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Andrey Ostrovsky - «Invertebrate life histories. Part 1»**

DAY 7 - Saturday - 18.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.00): **Andreas Wanninger - «Development of Bivalvia (Mollusca)»**

Practical session (10.00-13.00): **Andreas Hejnol and Andreas Wanninger** - development of bivalve *Mytilus edulis*, stimulation of spawning, artificial fertilization of gametes, observation and drawing of cleavage stages, onchostoma and veliger. Cultures set-up.

Practical session (12.00-13.00, parallel activity): continuation of projects on immunocytochemistry: embedding specimens.

Lunch (13.00 – 14.00)

Practical session (14.00-18.00): continuation working with cultures of *M. edulis* and other previously set cultures.

Practical session (15.00-18.00, parallel activity): continuation of projects on immunocytochemistry: CLSM session (individually with each pair of students).

Field excursion (18.00-20.00): collection of bryozoan colonies inhabiting intertidal zone in close vicinity of the WSBS; sorting animals and clutches in the laboratory

Dinner (20.00-21.00)

Lecture (21.00-22.30): **Andrey Ostrovsky - «Invertebrate life histories. Part 2»**

DAY 8 - Sunday - 19.06.2016

Breakfast (9.00-10.00)

Lecture (10.00-11.00): **Andrey Ostrovsky - «Reproduction and life cycles of Bryozoa. Part 1»**

Practical session (11.00-14.00): **Olga Kotenko - «Embryonic and postembryonic development of marine bryozoans Gymnolaemata»**, focus species: *Flustrellidra hispida*, *Cribrillina annulata*, *Celleporella hispida*, and larvae of *Electra pilosa* from plankton samples.

Lunch (14.00 – 15.00)

Practical session (15.00-18.00): working with earlier set-up cultures.

Practical session (15.00-18.00, parallel activity): continuation of projects on immunocytochemistry: CLSM session (individually with each pair of students).

Round table (18.00-20.00) : **all instructors** - tools of visualization of nervous system, including immunocytochemistry and cLSM

Dinner (20.00-21.00)

Lecture (21.00-22.30): **Alexander Ereskovsky - «Reproduction and ecology: introduction in marine invertebrates phenology»**

DAY 9 - MONDAY - 20.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.30): **Andrey Ostrovsky - «Reproduction and life histories of Bryozoa. Part 2»**

Practical session (10.30-13.00): **Olga Kotenko and Igor Kosevich - «Development of bryozoans Cyclostomata (Stenolaemata; Bryozoa). Polyembryony»**.

Lunch (13.00 – 14.00)

Practical session (14.00-20.00): working with earlier set-up cultures.

Practical session (14.00-20.00): continuation of projects on immunocytochemistry: CLSM session (individually with each pair of students). Discussion of results of immunocytochemical projects.

Dinner (20.00-21.00)

Lecture (21.00-22.30): **Grigory Genikhovich - «How an embryo makes its choices?»**

DAY 10 - TUESDAY - 21.06.2016

Breakfast (8.00-9.00)

Practical session (9.00-13.00): cLSM microscopy of the specimens stained with antibodies (students working by pairs)/ discussion of results of immunocytochemical projects/ rest of the students working with earlier set-up cultures

Lunch (13.00 – 14.00)

Lecture (14.00-15.00) **Grigory Genikhovich - «Mechanism of embryonic development»**

Practical session (15.00-18.30): **Grigory Genikhovich and Yulia Kraus** - Set-up of experiments on the pharmacological modulation of Wnt- FGF- and Nodal- signaling pathways in the representatives of cnidarians, annelids and molluscs + seastar (students work by pairs; one instructor per pair: **Grigory Genikhovich, Yulia Kraus, Andreas Hejnol, Denis Nikishin and Stanislav Kremnyov and others**).
Round Table (18.30-20.00): **all instructors** - nervous system formation in embryos and larvae of invertebrates
Dinner (20.00-21.00)
Lecture (21.00-22.00): **Grigory Genikhovich** - «How to establish, maintain and pattern body axes. Part 1»

DAY 11 - Wednesday- 22.06.2016

Field excursion (7.00-8.00): collection trip to Black Rocks for amphipods and isopods living at the intertidal zone
Breakfast (8.00-9.00)
Lecture (9.00-10.00): **Igor Kosevich** - «Development of Crustacea»
Practical session (10.30-13.00): **Igor Kosevich** - development of Crustacea, focus species: amphipods *Marinogammarus obtusatus*, *Caprella septentrionalis*, and various pelagic larvae.
Skill training (12.00-13.00): **Denis Nikishin** - time-lapse recording of development using embryos of amphipods and isopods.
Lunch (13.00 – 14.00)
Practical session (14.00-18:00): continuation experiments on signaling pathways (*G.loveni*, *O.limacina*, *L.saxatilis*)
Practical session (18.00-20.00): continuation working with crustacean cultures
Dinner (20.00-21.00)
Lecture (21.00-22.00): **Grigory Genikhovich** - «How to establish, maintain and pattern body axes. Part 2»

DAY 12 - Thursday - 23.06.2016

Field excursion (7.00-8.00): collection trip for nematodes living at the intertidal zone
Breakfast (8.00-9.00)
Lecture (9.00-10.30): **Vladimir Malakhov** «Early development of free living marine nematodes»
Practical Session (10:30-11:30): **Vladimir Malakhov** - set-up embryo cultures of nematode *Pontonema vulgare*
Practical session (11.30-13.00): **Igor Kosevich** - development of Crustacea, focus species: isopod *Jaera albifrons*.
Lunch (13.00 – 14.00)
Practical session (14.00-17.30): continuation experiments on signaling pathways (*G.loveni*, *O.limacina*, *L.saxatilis*)
Practical session (17.30-18.30): continuation working with crustacean cultures
Skill training (18.30-20.00): micromanipulation training in removing of fertilization envelope in sea urchin *Strongylocentrotus pallidus*
Dinner (20.00-21.00)
Lecture (21.00-22.00): **Igor Kosevich**: "Peculiarities of Module Organization in Hydrozoa»?

DAY 13 - Friday - 24.06.2016

Breakfast (8.00-9.00)
Lecture (9.00-10.00): **Denis Nikishin** - «Introduction to Deuterostomia. Development of Echinodermata. Radial cleavage»
Practical session (10.30-13.00): **Carmen Andrikou and Denis Nikishin** - development of *Strongylocentrotus pallidus* (sea urchins collected in advance by divers). Spawning stimulation, observation of fertilization and early development stages. Set-up cultures of sea star embryos.
Lunch (13.00 – 14.00)
Practical session (14.00-16:00): completion of experiments on signaling pathways (*G.loveni*, *O.limacina*, *L.saxatilis*)
Practical session (16:00-19:0): observation and drawing of echinoderm larvae from plankton samples (bipinnaria; echinopluteus and ophiopluteus)
Field excursion (19.00-20.00): collection sea stars *Asterias rubens* from the intertidal zone
Dinner (20.00-21.00)
Lecture (21.00-22.00): **Carmen Andrikou** - «Embryonic muscle development in echinoderms»

DAY 14 - Saturday - 25.06.2016

Breakfast (8.00-9.00)
Practical session (9.00-13.00): **Stanislav Kremnyov and Carmen Andrikou** - development of sea star *Asterias rubens*. Spawning stimulation, observation of fertilization and early development stages. Set-up cultures of sea star embryos. Dissection of viviparous sea star *Pteraster militaris*. Continuation observation of developing sea urchin embryos.
Lunch (13.00 – 14.00)
Practical session (14.00-16.00): **Grigory Genikhovich** - set up experiments on pharmacological modulation of the Wnt-, Nodal- и BMP- signaling pathways in sea star *Asterias rubens*.
Practical session (16.00-18.30): continuation observation of developing embryos of *Asterias rubens*.

Round table (18.30-20.00): **all instructors** - diversity of signaling pathways in different invertebrate lineages

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Andrey Ostrovsky** - «**Matrotrophy in invertebrates**»

DAY 15 - Sunday - 26.06.2016

Breakfast (9.00-10.00)

Day off. Excursion to Kos'yan Island. BBQ.

Practical session (18.00-20.00): continuation observation of developing embryos, continuation of experiments on signaling pathways (*A. rubens*)

Dinner (20.00-21.00)

Lecture (21.00-22.30): **Alexander Tzetlin** - «**Traditional fishery along the White Sea coast and recent state of the local resources exploitation**»

DAY 16 - MONDAY - 27.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.30): **Maria Semenova** - «**Development of Nemertea**»

Practical session (10.30-12.00): **Maria Semenova and Denis Nikishin** - Development of nemertean *Poseidon ruber*.

Practical session (12.00-13.00): observation of earlier set-up embryo cultures

Lunch (13.00 – 14.00)

Practical session (14.00-16.00): continuation of experiments on signaling pathways (*A. rubens*)

Field excursion (16.00-18.00): sponge field collection to the intertidal zone

Round table (18.00-20.00): **all instructors** - diversity of invertebrate larvae metamorphosis in different invertebrate lineages

Dinner (20.00-21.00)

Lecture (21.00-22.00): ...

DAY 17 - TUESDAY - 28.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.30): **Alexander Ereskovsky** - «**General characteristic of sponges with emphasis on their reproduction and development**»

Lecture (11.00-12.00): **Alexander Ereskovsky and Andrey Lavrov** - «**Methods for sponge collection, fixation, and experimental work with sponges**»

Practical session (12.00 - 13.00): **Alexander Ereskovsky and Andrey Lavrov** - set-up of experiments on reparative regeneration of *Sycon sp.* (collected by divers)

Lunch (13.00 – 14.00)

Field excursion (14.00-15.00): sponge collection at the intertidal zone

Practical session (15.00 - 18.00): **Alexander Ereskovsky and Andrey Lavrov** - set-up of experiments on reparative regeneration of *Leucosolenia complicata* and *Halisarca dujardini*. Set-up of experiments on cell dissociation and primmorphs formation of *Halisarca dujardini* and *Haliclona aquaeductus*

Practical session (18.00-20.00): completion of experiments on signaling pathways (*A. rubens*).

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Andreas Hejnol** - «**Current state of our knowledge of development of invertebrates**»

DAY 18 - Wednesday- 29.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.30): **Alexander Ereskovsky** - «**Comparative embryology of Porifera**»

Practical session (10.30-12.00): **Alexander Ereskovsky and Andrey Lavrov** - sponge anatomy and reproductive elements description, fixation, drawing.

Lunch (13.00 – 14.00)

Practical session (14.00-20.00): **Alexander Ereskovsky and Andrey Lavrov** - 1) results of experiments of primmorphs formation of *H. dujardini* and *H. aquaeductus*, 2) observation and drawing of larvae of *H. dujardini* and *Haliclona aquaeductus*, description of their behavior and metamorphosis, 3) continuation of experiments on reparative regeneration of *Sycon sp.*, *Leucosolenia complicata*, *Halisarca dujardini* (control, description, drawing, fixation).

Dinner (20.00-21.00)

Lecture (21.00-22.30): **Andrey Prudkovsky** – «**The biodiversity and seasonality of planktonic larvae of invertebrates of the White Sea**»

DAY 19 - Thursday - 30.06.2016

Breakfast (8.00-9.00)

Lecture (9.00-10.30): **Maria Semenova - «Development of Tunicates»**

Practical session (10.30-13.00): **Maria Semenova** - development of ascidians *Halocynthia pyriformis* and *Molgula retortiformis*, dissection of animals and observation and drawing of different developmental stages.

Lunch (13.00 – 14.00)

Practical session (14.00-16.00): observation of earlier set-up embryo cultures, observation of results of reparative experiments of sponges

Field excursion (16.00-17.30): for collection of hydroids at the Eremei rapids

Practical session (17.30-20.00): **Lev Belousov, Igor Kosevitch, Yulia Kraus and Mark Martindale** - diversity of realization of life cycles of hydrozoans *Clava multicornis*, *Dynamena pumilla*, *Laomedea flexuosa*, *Coryna sarsii*, inhabiting the Eremei rapids

Dinner (20.00-21.00)

Lecture (21.00-22.30): **Lev Belousov - «Morphomechanical approach to embryonic development»**

DAY 20 - Friday - 01.07.2016

Breakfast (8.00-9.00)

Practical session (9.00-13.00): last observations of all embryo cultures, finishing of preparation of drawings for a credit

Lunch (13.00 – 14.00)

Discussions (14.00-20.00): students and instructors discuss the results of the experimental projects (immunocytochemistry and signaling pathways) based on which students prepare oral presentations

Dinner (20.00-21.00)

Lecture (21.00-22.00): **Alexander Ereskovsky - Asexual reproduction in animals**

DAY 21 - Saturday - 02.07.2016

Breakfast (8.00-9.00)

Student presentations (9.00-13.00): oral presentation of the results of students' projects (pharmacological experiment and immunocytochemistry).

Lunch (13.00 – 14.00)

Excursion to the Olenevsky Island (14:00-21:00): examination of students' drawings and discussion problems of invertebrate development. Gala dinner at the island with picturesque view of the unique for Karelia sandy beach.

DAY 22 - Sunday - 03.07.2016

Breakfast (9.00-10.00)

Departure