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Using 3D reconstruction method in the investigations of Bivalvia larval development (by the example of *Hiatella arctica* L.)

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Bivalve larvae are one of the most important components of the planktonic community. The success of ecological and hydrobiological investigations depends to a large extent on a precise identification of these plankton animals. However, identification of bivalve larvae and early post-larvae has been extremely difficult. In spite of some classic descriptions of Bivalvia development and their larvae morphology (Lebour, 1938; Jørgensen, 1946; Rees, 1950; Lutz *et al.,* 1982) exact larval identification is still a laborious process.

Scanning electron microscopy is the most informative method of investigation of shell at present. But this method has some defects such as impracticability in field conditions and high cost. In using light microscope there is the danger of distortion during photography as a result of incorrect orientation of shell on glass slide.

All these complexities and apparent difficulties explain why complete descriptions of shell development for many species are absent. The present study deals with using of three-dimensional computer reconstruction in the investigations of larvae shells.

Material and methods

Investigations were carried out at the White Sea Biological Station of the Zoological Institute of the Russian Academy of Sciences. Larvae on a veliger and pediveliger stages were collected from plankton during June, July and August from various locations of the Chupa Bay of the White Sea. For a further development larvae obtained were cultured in polyethylene containers at a density of about 1-2 individuals per milliliter with recurrent aeration. Water temperature ranged from 11.0 to 12.5 °C and conformed to the temperature in the White Sea. Salinity was 25‰ and also conformed to the salinity in the sea. Larvae that reached a veliger stage were fed daily with *Dunaliella* sp. and *Isochrisis* sp. (Loosanoff & Davis, 1964).

When the larvae reached enough length they were immersed in detergent. Detergent dissolved soft tissue, but shells were remained intact. Molluscs were treated by detergent up to disintegration of the separate valves. Disarticulated valves selected for documentation of pattern of exterior surface were handled (mounted) on glass slide with the convexity up. Such orientation solved a problem of distortion during photography. Digital photography was carried out by using digital camera Nikon COOLPIX 4500 and microscope MBB-1 A. Microphotos were obtained top-down with fixed step of focus. Photos are generally taken with a x40 objective and x10 ocular, giving x400 enlargements. For each valve a series of photographs (from 10 to 20 in most cases) corresponding to the different depths of sharpness were obtained.

Each photo from the series contained ring of sharpness corresponding to the shape of section on given level (Fig. 1).

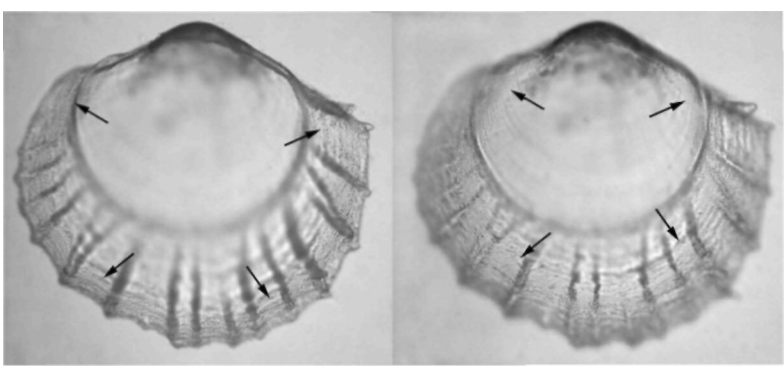

Fig. 1. Microphotographs of the post-larval *Chlamys islandicus* containing rings of sharpness. Arrows mark the edges of sharpness

Obtained series of microphotographs were processed by computer. Using particularized software all unnecessary information was removed from the each microphotograph in order to single out only the ring of sharpness (Fig. 2, *A).* Series rings of sharpness, corresponding to a valve sections used as a base for creation of a three-dimensional model of shell.

Construction of the model of shell was implemented with software designed for histological and medical three-dimensional reconstruction *(http://www.ablesw.com/3d-doctor/; http://www.sph.sc.edu/comd/rorden/; http://rsb.info.nih.gov/ij/; http://www.sim.hcuge.ch/uin/).* This software allowed creating a raw model of the valve (Fig. 2, *B).* The raw model was exposed to further processing. The artifacts that originated during the re-

construction, were removed using 3D graphic software. Editing was controlled according to the microphotographs. The last phase of reconstruction was smoothing of the object. Post-production of the computer model of shell was texturing of surface (Fig. 2, *C*). Texture and bump maps were created by imposition of images from different depths of sharpness (Baker, 2001). For creating of the object's texture maps and bump maps the same series of microphotographs were used as well as for creating a 3D model.

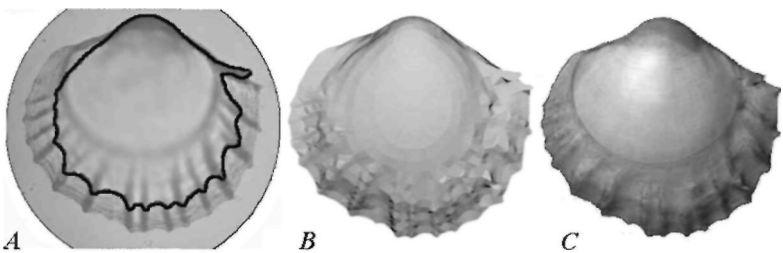

Fig. 2. Main steps of the larvae shell reconstruction. *A* - microphotograph with selected section profile; *B* - raw non-edited model; *C* - edited and textured model

If necessary, these operations were repeated for reconstruction of another valve, and both valves were combined into an united object.

Any manipulations in three-dimensional virtual space and any measurements are available f o r obtained model of a shell.

Results and discussion

In this paper we illustrate ability of our method by the example of *Hiatella arctica*, which is a common species in the White Sea fouling communities and often plays an important role in benthic assemblages. First descriptions of *Hiatella* larvae can be found in the papers of Odhner (1914), Thorson (1936), and Lebour (1938). In her references, Lebour stated that larvae of this species are some of the commonest and largest veligers in the plankton both inside and outside the breakwater at Plymouth. Nevertheless, *Hiatella* larvae have not been identified with certainty in plankton samples. Lebour (1938) and Jørgensen (1946) think that this is due to the very close resemblance of *H. arctica* to veliger of *H. gallicana*. But, as was already noted, the identification of bivalve larvae and early post-larvae is extremely difficult.

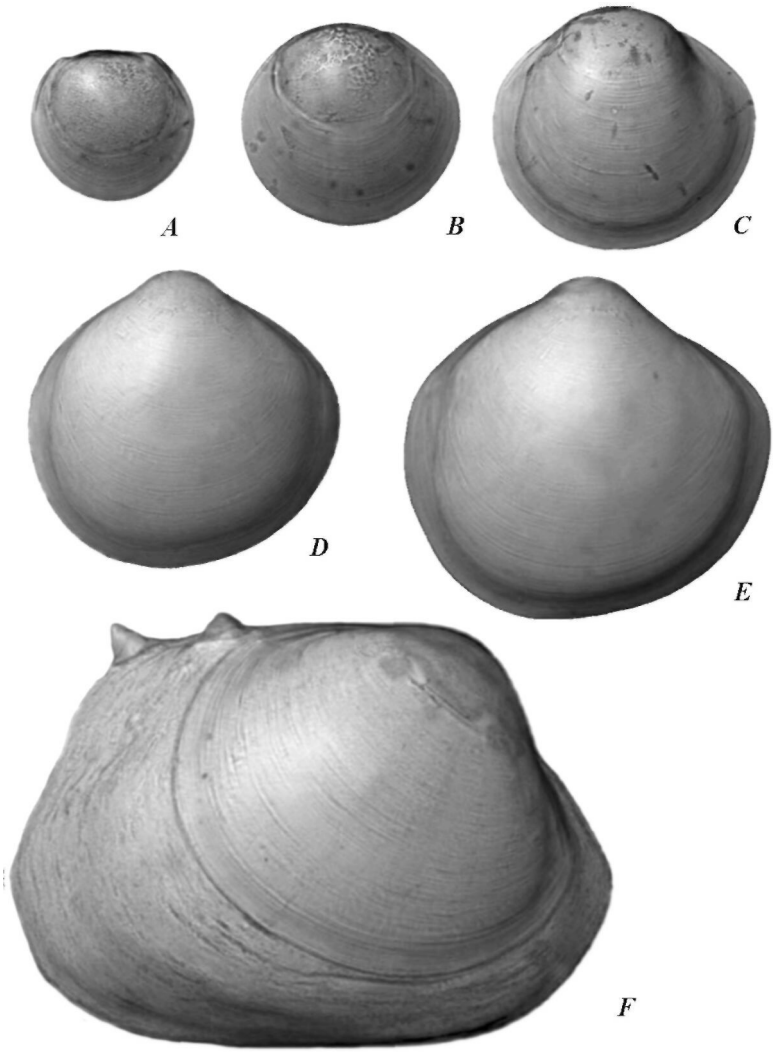

Fig. 3. Development of the *Hiatella arctica* shell from the straight hinge stage to post-metamorphosis mollusc. *A* - straight hinge stage (120 μm); *B* - umbo forming (160 μm); *C* - early pediveliger (220 μm); *D* - pediveliger (310 μm); *E* - pre-metamorphosis pediveliger (400 μm); *F* - post-metamorphosis *Hiatella arctica* (750 μm)

These bivalves spawn over the whole summer, from the middle of June through September. Larvae were present in plankton from June through November. It is a considerably longer period than Kaufman (1977) stated. Early development from cleavage to veliger stage was similar to that described by Malakhov and Medvedeva (1985) and Flyachinskaya and Kulakovsky (1991) for *Mytilus edulis*.

The smallest straight hinge (prodissoconch-I) veliger observed had length and height 120 and 90 μm, respectively (Fig. 3A). At this early stage we are yet able to observe star zone of the shell, produced by the shell gland. The shell is usually yellowish pink.

As shell length reached 160 μm, the hinge-line was obscured by the appearance of a low, rounded umbo (Fig. 3B). Along with star zone appeared a radial zone produced by mantle. An expressed umbo appeared at a shell length between 160 and 200 μm (prodissoconch-II) (Fig. 3C). The color of shell on this stage was yellowish pink too.

The veliger having reached 300 μm is one of the most easily recognizable in the plankton because of somewhat triangular shape. The anterior end of the shell is more pointed than posterior, and the whole shell is triangular with the longest side ventrally (Fig. 3D). The mantle line is distinctly expressed and envelopes almost the whole shell. All larvae collected from plankton and larvae reared in laboratory were metamorphosed at a size of approximately 380-400 μm (Fig. 3E). Color of shell changed to dirty yellow with pinkish boundary.

Dramatic changes in shape and structure occur in the shells of many species of bivalves at the time of settlement. After metamorphosis, at a length of 500 μm *H. arctica* shell has the same shape of prodissoconch, but the emerging dissoconch starts to grow toward siphonal edge (Fig. 3D). There are two or more posterior dorsal spines on the dissoconch, which are a typical feature of *Hiatella* spat. Adult shell usually is almost white.

In *Hiatella arctica* a pronounced boundary separates the smooth exterior surface of the prodissoconch and the distinct, co-marginally ridged surface of the dissoconch (Fig. 3D).

The shape of larvae that reached 200-500 μm closely agrees with description of *Hiatella arctica* development given by Lebour (1938) and Jørgensen (1946) and Manushin (1998). However, the present study is the most detailed description of *Hiatella arctica* shell from the straight-hinge larvae to post metamorphosis mollusc.

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