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Original Article

Morphological development and allometric growth patterns of *Acipenser persicus* Borodin, 1897 (Actinopterygii, Acipenseridae) during early development

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Abstract: Morphological development and allometric growth patterns of reared Persian sturgeon, *Acipenser persicus*, were studied from hatching to 50 days post-hatching (dph). The larvae were sampled, their left sides photographed and seven morphometric characters, including total length, head length, tail length, trunk length, snout length, caudal peduncle and predorsal length were measured. Allometric growth patterns were calculated as a power function of total length and described using the growth coefficient to find important steps in early life history. The total length of the newly hatched larvae and fry were 10.59±0.8 and 38.8±2.9 mm at 1 and 50 dph, respectively. Morphogenesis and differentiation were the highest rates during the first 11 days of early development, i.e. endogenous feeding period. There were higher growth rate of head, snout and tail regions compared with those of other organs from the hatch up to yolk sac absorption, followed by positive or almost isometric patterns, after the begin of exogenous feeding, showing priority to enhance the feeding and swimming capabilities. This study confirmed that most of morphological changes of this species are occurred from hatching until the onset of exogenous feeding i.e. during the lecithotrophic phase.

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Introduction

Fish larvae experience complex processes of morphogenesis and differentiation during early ontogeny by changing their morphology, metabolism rate, swimming ability and behaviour (Osse and Van den Boogart, 1995; Van snik et al., 1997; Gisbert, 1999, 2014; Koumoudouros et al., 1999). The alterations of body shape with increasing size (Osse and van den Boogart, 1995; van Snik et al., 1997) as a result of different relative growth patterns of the body parts has been defined as allometry (Fuiman, 1983). Allometry is a common feature during early larval development, ensuring the development of the most essential organs for primary functions, followed by the development of organs with lower priority for survival (Osse and van den Boogart, 1995; Hasanpour et al., 2015a).

During the short period of early development, fish larvae are extremely vulnerable and subjected to various environmental factors (Claramunt and Wahl, 2000; Hardy and Litvak, 2004). Since the alteration of body shape is occured in response to environmental conditions (Dettlaff et al., 1993; Eagderi et al., 2015) and poor environmental conditions may cause deformities during early ontogeny resulting negative effects on growth performance and survival of reared fish larvae. Therefore, information about the early morphological development and allometric growth patterns in the farmed fishes is crucial for their proper managment in aquaculture industry (Chatain, 1994; Koumoundoura et al., 1994, 1999; Bengtson, 1999; Hasanpour et al., 2015b; Asgari et al., 2013a, b, 2014). Furthermore, understanding normal growth pattern during early development will help to identify abnormal deformities and improving the quality of produced fish by improving environmental conditions (Segner et al., 1995; Koumoundoura et al., 1994, 1999; Bengtson, 1999).

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Persian sturgeon, *Acipenser persicus*, is an anadromous sturgeon distributed along the Iranian coastal of the Caspian Sea (Birstein et al., 1997). The artificial propagation and rearing of this species has been developed since 1971 for restocking the natural populations or production of marketable-size fish (Chebanov and Billard, 2001). Hence, information about the growth patterns of this species fish may allow better understanding the biological process in the early life stages and their priorities during early growth. Such information provides insight into fish biology, behaviour, ecology and especially aquaculture to promote the development of optimal rearing protocols and improve the quality of juveniles (Koumoundouros et al., 1999; Çoban et al., 2009) and evaluate the appropriateness of produced fish for restocking or further rearing (Gisbert et al., 2002). Therefore, in the present study, Persian sturgeon, *A. persicus*, larvae and juvenile specimens were studied from hatching up to 50 days post hatching (dph) to describe its early morphological development and allometric growth pattern.

Materials and Methods

The larvae were obtained from artificial propagation of a hormonally-induced (LHRHa2) female Persian sturgeon fertilized with the milt of three males at the Shahid Rajaei propagation facility (Sari, Mazandaran Province, Iran) in 2015. Eggs were incubated in the Yushchenko incubators at 11-12°C (750 gram egg per unit) and hatched after about 1800 degree/day (6 day). The newly-hatched larvae were allocated to three 500-L circular fiberglass tanks connected to a flow-through freshwater system. During the larval rearing period, water temperature, dissolved oxygen and pH were 15.9±0.6°C, 7.1±0.6 mg.L^{-1} and 7.2±0.1, respectively. The larvae were reared under natural photoperiod.

The larvae were fed with a mixture of *Artemia* nauplii and cladocerans (*Daphnia* sp.) from 8-25 days post hatching (dph) (500 larvae/day–6 times a day). Then, a short co-feeding phase based on cladocerans and an inert diet (Biomar, Denmark; D_1 - particle size=0.5) was conducted from 25 to 30 dph, at the rate of 20% of stocked fish biomass 6 times a day. The food particle size were progressively adjusted according to the fish size. All tanks were cleaned and the bottom of the tank siphoned to remove uneaten feed and faces three times a day.

Specimens were sampled from hatching till 10 dph, every day, up to 20 dph, every two days and afterwards, at 25, 30, 40 and 50 dph. Ten specimens were randomly sampled for different stages using a scoop net, anaesthetized using 1% clove oil and weighed (BW, to the nearest 0.01 mg) using an analytical microbalance. The left sides of the specimens were photographed using a stereomicroscope equipped with a digital Cannon camera with a 5 MP resolution. Then the larvae were preserved in 5% buffered formalin and stored in 70% ethanol after 24 hours.

Seven morphometric characteristics including total length (TL), head length (HL), tail length (TaL), trunk length (TrL), snout length (SnL), caudal peduncle (CP) and predorsal length (PdL) were measured using the software imageJ (version 1.240). All characteristics were measured along the lines parallel or perpendicular to the horizontal axis of the body. The allometric growth patterns were calculated as a power function of total length using non-transformed data: $Y= aX^b$, where Y is the independent variable; X, the dependent variable, a, the intercept and *b*, the growth coefficient. Isometric growth, positive and negative allometric growth are indicated by b=1, b>1, b<1, respectively (Van snik et al., 1997). Robustness of the regression was examined using r^2 and its significance level. The inflexion points of growth curves were determined according to Fuiman (1983) and Van snik et al. (1997). Graphs were drawn and data were analysed using Excel 2013 (Microsoft Corporation) and Past (version 2.17).

Results

Morphological development: At hatching, total length and weight of the larvae were 10.59±0.8 mm and 26.7±0.5 mg, respectively, and increased to 38.8±2.9 mm and 2.1±0.8 g at 50 dph. At hatching, the mouth and gill slits were closed and tail was as proterocercal

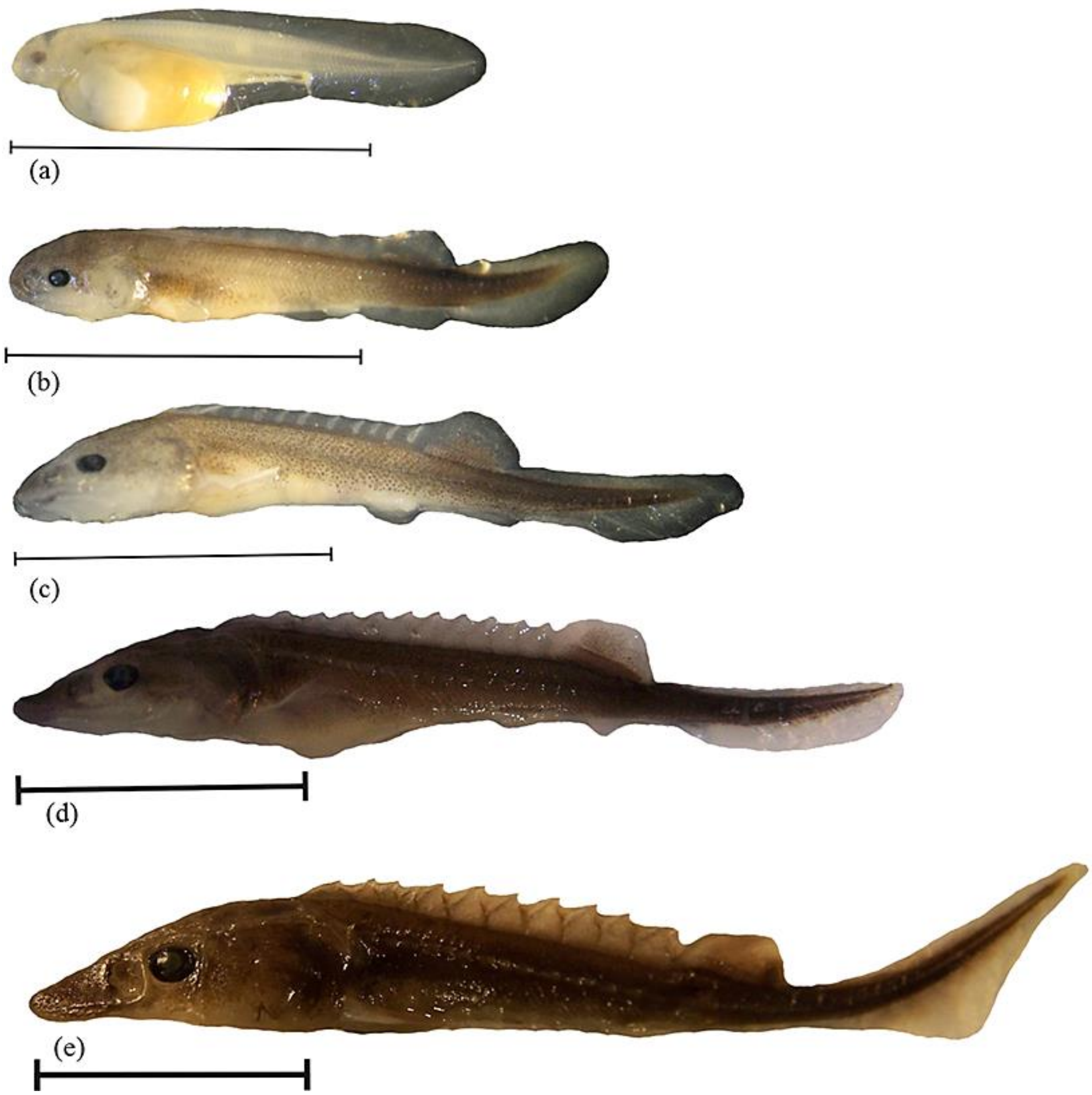

Figure 1. Early life stages of *Acipenser persicus*. (a) 2 dph, (b) 11 dph, (c) 17 dph, (d) 28 dph and (e) 50 dph (the scale bars indicate 10 mm).

and bordered by a wide primordial fin-fold. At this time, the notochord was visible, eyes were pigmented and olfactory organ was observed as an external opening. At 2 dph, the pectoral fin appeared, mouth was open with few taste buds around it, gill slit was open and its filaments were visible inside the branchial cavity (Fig. 1a). At 3 dph, the eyes were darkly pigmented, size of the pectoral fin increased, melanin plug was appeared, yolk sac was divided into two parts, upper and lower lips were appeared as skin fold around the mouth silt, and a small barble was observed.

At 4 dph, the length of barble increased and fin folds became wider in their base with clear pigmentation. At 5-6 dph, the ventral fin appeared and rays of the dorsal and anal fins were observed. In addition, the body pigmentations increased with dense pigmentation on the head and tail regions. Larvae possessed about 10 canine teeth on the upper and lower jaws at 7-8 dph. At this time, the feeding apparatus for grabbing food items along with related sense organs *e.g.* taste buds and eye were developed for exogenous feeding. In addition, the dorsal and anal fins were clearly distinguishable from surrounding fin folds and rays of the paired fins were observed. At 9-10 dph, external yolk sac was almost depleted and food items were observed inside the digestive system of the most larvae and gill arches were completely covered with opercle. The rate of changes in external morphology was decreased after 11 dph. The first

Table 1. a, the intercept; *b*, the growth coefficient and r^2 correlated coefficient of different body segments before and after inflexion point of *Acipenser persicus* (*P<0.01).

Characters	Before inflection point				After inflection point			
	b	**a**	r^2	**P- value**	***b***	**a**	r^2	***P*- value**
Head length	1.90	0.13	0.92	*	1.85	0.22	0.91	*
Caudal peduncle	1.18	0.07	0.63	*	0.68	0.09	0.71	*
Trunk length	0.23	0.57	0.25	*	0.85	0.37	0.85	*
Snout length	1.7	0.05	0.8	*	1.38	0.07	0.86	*
Tail length	1.43	0.31	0.95	*	0.97	0.40	0.89	*
Pre-dorsal length	0.20	0.22	0.01	*	1.36	0.15	0.91	*

scutes were dorsal and ventral scutes that appeared at 11 dph. Between 18-19 dph, lateral and ventral scutes were developed as some rows of small spines with their differentiation were completed during 0-40 dph in an anterior-posterior direction (Fig. 1d, e). At 35-40 dph, remaining of the fin folds were disappeared completely and larvae were transformed as miniature shape of adults in the external morphology.

Allometric growth pattern: Based on morphological development and growth coefficients, life stages of Persian sturgeon from hatching (10.59±0.08 mm TL) up to 50 dph (38.8±2.8 mm TL), could be divided into two distinct phases, including larval and post-larval stages. The larval stage starts from hatching up to onset of the exogenous feeding at 11 dph (11.6-18.7 mm in TL) and the post-larval stage continues till metamorphosis (18.9-38.8 mm in TL).

The growth pattern of all body segments can be divided into two phases (Fig. 2 and Table 1). The HL and SnL had strongly-positive allometric growth in relation to TL during whole early developmental stage with inflection points at 13 dph (*b*=1.9 and 1.23) and 12 dph (*b*=1.7 and 1.41), respectively. The growth patterns of the tail region including CP and TaL were positive allometric prior to their inflection points at 5 dph (*b*=1.18; 15.7 mm in TL) and 11 dph (*b*=1.43; TL=18.6 mm in TL), respectively, whereas TaL showed a relatively-isometric (*b*=0.97) and CP, negative (*b*=0.68) allometric growth patterns during the flexion stage. There were negative allometric patterns for TrL and PdL during the flexion stage at 11 dph (*b*=0.23; 18.6 mm in TL) and 4 dph (*b*=0.20; 14.1 mm in TL), respectively. There were relatively negative (*b*=0.85) and positve (*b*=1.36) growth patterns for TrL and PdL, respectively, during the post-flexion period (Fig. 2, Table 1).

Discussion

The organs developed and morpho-anatomical characteristics changes in a stepwise fashion, which is regulated by gene expression and influenced by environmental parameters (Gilbert and Bolker, 2003; Osse and Van den Boogart, 1995). At hatching, most of the functional systems in Persian sturgeon larvae were incomplete. Thus, these larvae require to develop quickly the most essential organs for primary functions during the early development to survive (Dettlaff et al., 1993; Gisbert, 1999). This changes are accompanied with morphological alternations that is led to allometric growth patterns (Gisbert and Doroshov, 2006).

Based on the results, the growth pattern of the head in *A. persicus* was positive during early ontogeny, whereas it was divided into two distinct phases in *A. baeri* with different growth patterns (Gisbert, 1999) *i.e.* during first phase, endogenous feeding was positive followed by isometric pattern during exogenous feeding (Gisbert and Doroshov, 2006). However, this difference may be due to their snout form and swimming hydrodynamics (Gisbert and Doroshov, 2006). Positive allometric growth of the head is a common phenomenon during early ontogeny of many fish species, *e.g.* sturgeons (Van Snik et al., 1997). The positive growth pattern of head can show the developmental priority of this organ during early developmental stage because it is related to vital functions such as branchial respiration, brine development and feeding apparatus (splachno-

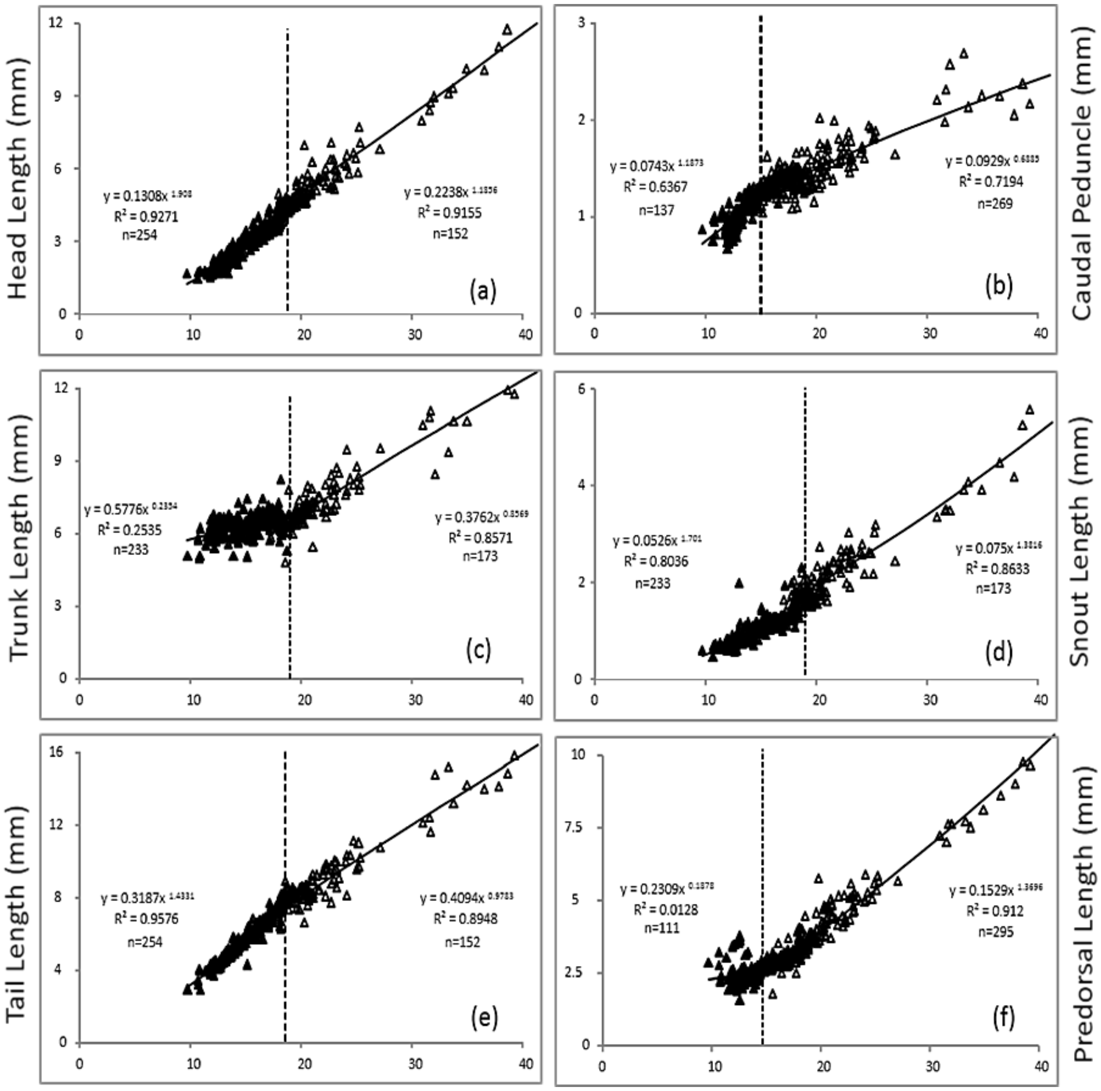

Figure 2. Growth allometries of the different body segments in *Acipenser persicus* (R^2=correlated coefficient).

cranium) (van Sink et al., 1997; Gisbert, 1999; Gisbert et al., 2002; Osse and van den Boogart, 2004). In addition, rapid growth of the head length leads larvae to have an efficient feeding system to capture the higher food particles which have more energetic than smaller one (Osse et al., 1997; Van Sink et al., 1997; Mathias and Li, 1982). Differentiation of the sensory structures i.e. barbles equipped with taste buds and eyes make larvae to be independent from yolk sac as an endogenous feeding (Gisbert, 1999).

The growth pattern of the snout was positive during all period with a flexion point at 12 dph similar to that of *A. baeri* (Gisbert, 1999) and this growth pattern has been reported for many fishes (Strauss, 1995; Schmidt, 2001; Geerinckx et al., 2008). The developed sensory systems such as taste buds and sensory chanells on the ventral face of the sturgeon's snout can play a vital role for feeding (Geerinckx et al., 2008).

The negative growth pattern of the trunk from hatching up to 12 dph was similar to those of other fishes (Osse and van den Boogart, 2004), whereas it changed from 12-50 dph to relatively isometric pattern. Similar result reported for *A. medirostris* (Gisbert and Doroshov, 2006), whereas in *A. baeri*, it was negative during whole developmental stages (Gisbert, 1999).

The positive allometric growth pattern of the tail in Persian sturgeon from hatching up 13 dph may reflect its priority after development of the head that is related to vital functions such as feeding and swimming ability (Osse and van den Boogaart, 2004) and changing swimming mode that can have an important role for locomotion (Osse et al., 1997). Similar results have been reported in other sturgeons e.g. *A. baeri* and *A. medirostris* (Gisbert, 1999; Gisbert and Doroshov, 2006). Since predation and starvation are main causes of mortality in fish larvae, the early development of feeding apparatus and swimming organs appear to have priorities during the early ontogeny (Osse et al., 1997). There was positive growth pattern in the caudal peduncle of *A. persicus* up to 5 dph and then altered to negative. This pattern was positive in *A. medirostris* and isometric in *A. baeri* (Gisbert, 1999; Gisbert and Doroshov, 2006). Positive allometric growth pattern in anterior and posterior section of larvae body before complete development may reflect the larvae adaptation to decrease costs of transport (Van Snik et al., 1997). Therefore, faster growth patterns of the head and tail regions would be a favorable condition for decreasing the stop pressure on the larvae body (Osse and Van den Boogart, 1995) as seen in *A. persicus*.

This study showed that important morphological and morphometric modifications occurred during early life stages of Persian sturgeon holding valuable information on changes in functional demands throughout ontogeny. Based on our results, most of morphological changes of this species, during the lecithotrophic phase, are occurred from hatching until the onset of exogenous feeding, *i.e.* 12 dph and after onset of exogenous feeding until 50 dph.

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چکیده فارسی

توسعه ریختی و الگوی رشد آلومتری در تاسماهی ایرانی، *Asipenser persicus* Borodin, 1897 (Actinopterygii, Acipenseridae) در مراحل اولیه تکوین

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چکیده:
در این تحقیق، توسعه ریختی و الگوی رشد آلومتری در تاسماهی ایرانی، *Asipenser persicus* پرورش داده شده از زمان تخم‌گشایی تا پنجاه روز پس از آن مورد مطالعه قرار گرفت. پس از نمونه‌بردای لاروها، از نیمرخ چپ آنها عکس‌برداری شد و ۷ ویژگی ریختی شامل طول کل، طول سر، طول دم، طول تنه، طول پوزه، طول ساقه دمی و طول پیش پشتی در آنها مورد سنجش قرار گرفت. الگوهای رشد آلومتری به‌صورت تابع توانی طول کل و براساس ضریب رشد، برای پیدا کردن مراحل مهم در طی فرآیند اولیه چرخه حیات، توصیف شد. در روزهای ۱ و ۵۰ پس از تخم‌گشایی طول کل لاروهای تازه تفریخ شده و بچه ماهیان به‌ترتیب ۲/۹± ۳۸/۸ و ۰/۸±۱۰/۵۹ میلی‌متر بود. ریخت‌زایی و تمایز در طی ۱۱ روز اول پس از تخم‌گشایی، به عبارت دیگر در دوره تغذیه داخلی، دارای نرخ بالایی بود. از زمان تخم‌گشایی تا جذب کامل کیسه زرده، بخش‌های سر، پوزه و دم در مقایسه با سایر بخش‌ها دارای نرخ رشد بیشتر بودند که با الگوی رشد آلومتری مثبت یا ایزومتری پس از شروع تغذیه خارجی دنبال شد که اولویت افزایش توانایی تغذیه و شنا را نشان می‌دهد. این مطالعه تایید کرد که بیشتر تغییرات ریختی این گونه از زمان تخم‌گشایی تا اولین تغذیه خارجی یعنی دوره جذب کیسه زرده به وقوع می‌پیوندد.

کلمات کلیدی: انتوژنی، ریخت‌زایی، ریخت‌سنجی، دریای خزر.