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НАУЧНЫЕ СООБЩЕНИЯ

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IDENTIFICATION OF SOME COMMON FOOD ITEMS IN THE GUTS OF FISH LARVAE AND JUVENILES IN THE BLACK SEA

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Over the past decade the positive trends in the average annual number of fish larvae and in the copepod population dynamics in the coastal area of the Black Sea agree. The increased fish larvae abundance is hypothesized due to improvement of their nutrition associated with the drastic increase in number of introduced invasive cyclopoid copepod *Oithona davisae*. This assumption is difficult to be verified through fish gut content analysis in absence of methodology allowing prey species identification from their fragmentary residual remnants. Our paper offers an original approach to identification of several common copepod prey using specific distinctive features detected on their chitin fragments from guts of fish larvae and juveniles. To identify specific features of the common species from the coastal areas off Sevastopol (*Acartia tonsa*, *Oithona davisae*, *Longipedia* sp., *Cyclopina* sp.), alive copepods were isolated from the samples and reared as monospecific cultures in laboratory. Images of alive copepods of each species at successive stages of development and their moulted exoskeletons were compared with the images of chitin remnants found in the fish guts. This technique discloses relatively intact specific morphological features remaining undigested in chitin fragments of prey. These species-specific taxonomic features are suggested to be used for trophic analysis of the Black Sea fishes at early stages of development. Application of proposed method is helpful for assessment of qualitative and quantitative composition of consumed prey and selectivity of fish, especially during the changes in zooplankton community structure affecting significantly survival of fish generations.

Ключевые слова: food items, fish larvae, fish juveniles, copepods, *Oithona davisae*, Black Sea

Numerous studies have shown that small copepods at all stages of their development are the initial and the main prey of fish larvae [3, 16]. The positive trend of average annual number of fish larvae in the Black Sea coastal waters is observed last decade and corresponded to positive trend in copepod population dynamics [9]. The increase of fish larvae abundance in the Black Sea is assumed to be related to the improvement of their food web associated with the introduction of a new species of cyclopoid copepods, *Oithona davisae* Ferrari & Orsi, 1984, which currently dominate in zooplankton community in the second half of the year. Verification of this hypothesis through studying of gut contents of fish larvae and juveniles is complicated because the food items are changed significantly during digestion. Various chitinized cuticular processes on copepod carapace and limbs such as spines, setae etc. used as identification keys for copepods, are often not found after digestion. The only known special guide for identification of prey items by their fragments from the guts exists only for the fresh-water organisms [2] while none was found for marine fish and their prey. The lack of methodological base for identification of food items by their residual fragments complicates the taxonomic identification of consumed items, and makes it difficult to assess the contribution of different prey species to feeding of fish at early stages. The goal of our work is to propose our own approach to find specific features on chitin fragments of several common copepod species (needed for identification of their chitin fragments from fish larvae guts) by comparison of alive prey and their *post-mortem* analogues from fish guts. In particular, to assess the importance of different stages of *Oithona davisae* in the fish diet it was necessary to determine specific identification features for this copepod species different from other common copepod species found in the fish guts.

MATERIAL AND METHODS

Fish were sampled during the warm periods in 2013 and 2014. Mugil juveniles were caught in the open sea by neuston net in August, 2013, and with a dip net in a shallow coastal area of Sevastopol Bay in August — September. Other species of fish larvae were caught in August, 2014 by both neuston and Bogorov — Rass ichthyoplankton nets.

Feeding of fish larvae and juveniles was studied after method [7]. Samples were preserved in a 4 % solution of formaldehyde. Identification of fish larvae species was carried out according [6]. Identification of food items in fish guts was carried out by [10, 11] with the exception of invasive cyclopoid copepod *Oithona davisae*; for identification of copepod stages of this species was carried out by [8] and nauplii by [14].

Study of fish feeding was based on analysis of guts contents of analysis was carried out for 63 specimens of mugil juveniles and 227 specimens of fish larvae from Engraulidae, Mugilidae, Atherinidae, Carangidae, Sciaenidae, Sparidae, Mullidae, Labridae, Blenniidae, Gobiidae, Callionymidae families. Larvae were dissected under a binocular microscope, guts were separated from the larvae body and thereafter the guts were cut off with two thin needles, and the gut contents removed to a drop of fresh water with addition of glycerine on a glass slide and examined under microscope. Food items found in guts were measured under Nikon Eclipse 200 at a magnification × 100 and × 400 and identified to maximum possible taxon.

After complete digestion of copepods their chitin exoskeleton, or at least some of its identifiable components are usually severely damaged. Therefore, it was necessary to highlight the most typical specific details of chitin exoskeleton left even on deformed remnants which allow identification of the prey taxon. For comparison and analysis of food items and their fragments in the guts, the most common copepods species were selected: *Oithona davisae* (copepodite and nauplii), *Acartia clausi* Giesbrecht, 1889 + *A. tonsa* Dana, 1849 (nauplii), *Cyclopina* Claus, 1886, sp. (copepodite and nauplii), *Longipedia* Claus, 1883, sp. (nauplii).

To identify specific features of the common copepods from Sevastopol coastal areas (*Acartia* sp., *Oithona davisae*, *Longipedia* sp., *Cyclopina* sp.), these copepods were isolated from alive zooplankton samples, and reared as monospecific cultures in laboratory conditions. Additionally, for comparison, alive marine Cladocera were sampled from the field.

Digital images of alive copepods from laboratory cultures at successive development stages and their moulted exoskeletons were compared with the images of chitin remnants found in the fish guts were taken with a camera Ikegami ICD-848P attached to the inverted microscope Nikon Eclipse 200, at magnification × 100 and × 400. The images were processed in the program ImageJ 1.45s (National Institutes of Health, USA) [4].

RESULTS AND DISCUSSION

Currently copepods of *Oithona* genus in the Black Sea are represented by two species: invasive *Oithona davisae* and aboriginal species *Oithona similis* Claus, 1866. The latter species as opposed to thermophilic *O. davisae* inhabit the upper sea layers only during cold season [5], and therefore, may be excluded from the possible fish prey during the warm season. In addition, *O. similis* is about 1.3 times larger and has no rostrum, which is characteristic of invasive species *O. davisae* [8, 15].

Specific features of females and copepod stages of *O. davisae*. Among the studied food items *O. davisae* late copepodite and adult female stages can be easily identified due to their sharply curved rostrum

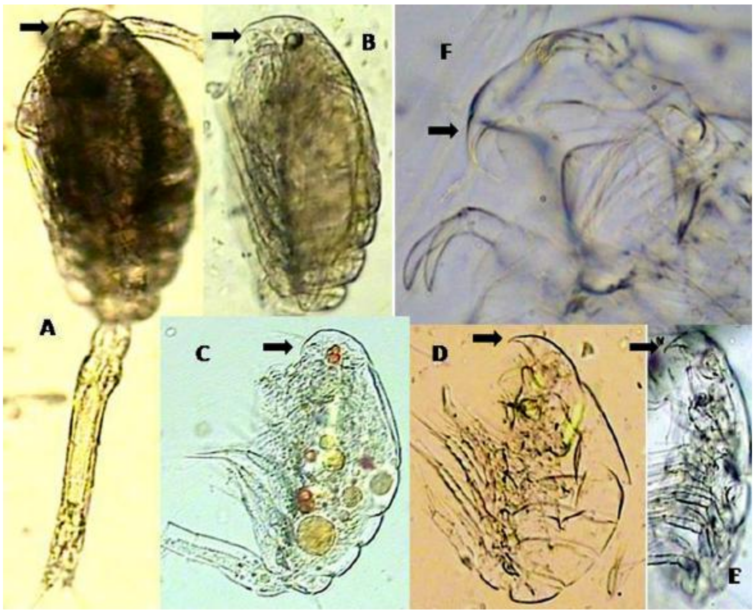

Figure 1. Females of *Oithona davisae*: A — alive specimen from the culture; B–E — specimens from fish guts: B — from the foregut, start of digestion, CT 0.3 mm; C — from the midgut CT 0.23 mm; D — completely digested copepods — chitin remnants of CT 0.3 mm and E — completely digested specimen from the hindgut, deformed and squashed CT 0.3 mm; F — completely digested copepod, head area. Arrows point to the rostrum of females; CT — cephalothorax. Photo: 1A–1E — magnification × 100; 1F — magnification × 400

Fig. 1. Самки *Oithona davisae*: A — живая особь из культуры; B–E — особи из кишечников личинок рыб: B — из верхнего отдела кишечника, начало переваривания, ЦФ 0.3 мм; C — из средней части кишечника, ЦФ 0.23 мм; D — полностью переваренная копепода, остатки хитинового покрова, ЦФ 0.3 мм; E — полностью переваренная копепода из нижнего отдела кишечника, сплющенный ЦФ 0.3 мм; F — полностью переваренная копепода, остатки хитинового покрова, голова. Стрелки указывают на роструум самок *O. davisae*; ЦФ — цефалоторакс. Фото: 1A–1E — при увеличении × 100; 1F — при увеличении × 400

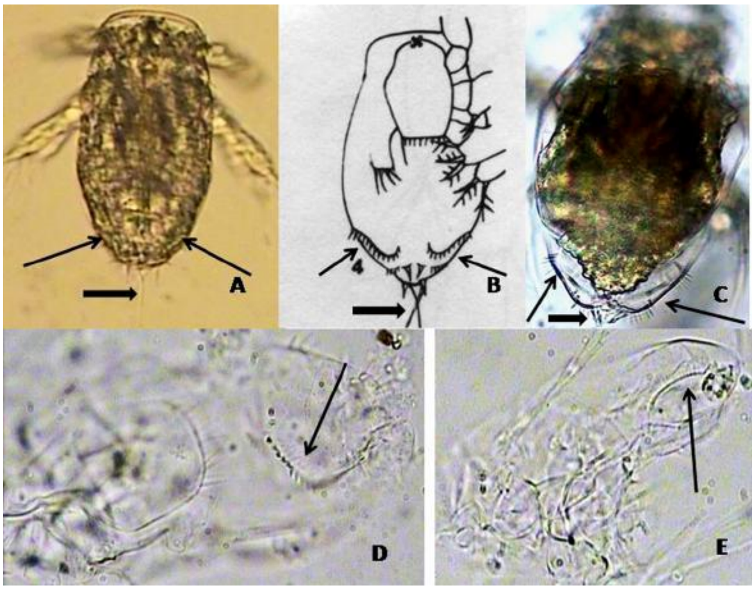

Figure 2. Nauplii of *Acartia* spp.: A — alive specimen from the culture; B — nauplius, drawing from [11]; C — alive specimen with focus on its ventral setae; D and E — remains of chitin exoskeleton of completely digested nauplius from the hindgut. Thin arrows point to the ventral setae of *Acartia* nauplius. Thick arrows point to the long caudal setae of *Acartia* nauplius. Photo: 2A–2C — magnification × 100; 2D, 2E — magnification × 400

Fig. 2. Науплиусы *Acartia* spp: A — живая особь из культуры; B — науплиус, рисунок из [11]; C — живая особь с фокусом на ряды вентральных щетинок; D и E — остатки хитинового покрова полностью переваренных науплиусов из нижней части кишечника. Тонкие стрелки указывают на ряды вентральных щетинок у науплиусов *Acartia*. Толстые стрелки указывают на длинные каудальные щетинки у науплиусов *Acartia*. Фото: 2A–2C — увеличение × 100; 2D, 2E — увеличение × 400

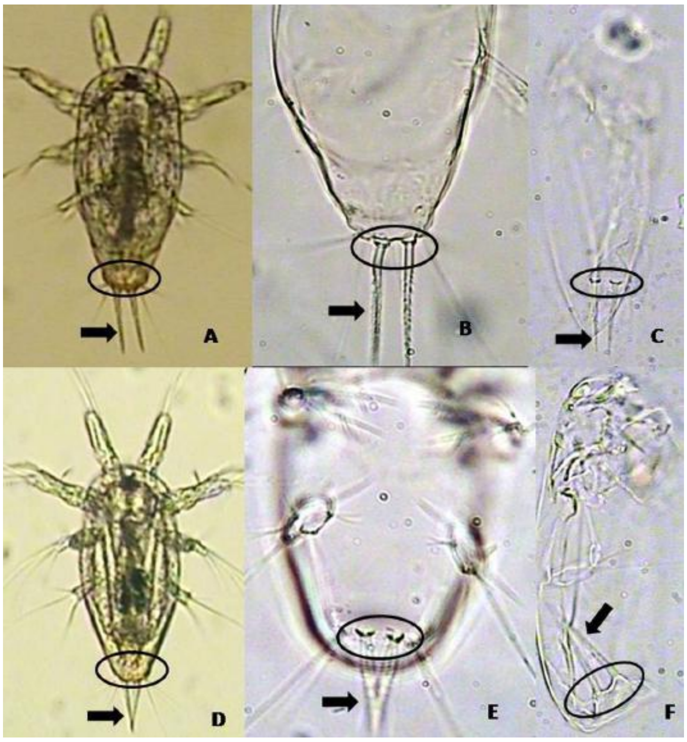

Figure 3. Nauplii of *Oithona davisae*: A–C — specimens with caudal setae arranged parallelly; D–F — specimens with crossed caudal setae; A and D — alive specimens from the culture; B and E — chitin exoskeleton remains of completely digested nauplius from the midgut; C and F — deformed chitin exoskeleton of completely digested nauplius from the hindgut. Identification characteristics: 1) arrows point to pair of caudal spines; 2) species-specific attachment shape of caudal spines is showed by ellipse. Photo: 3A and 3D — magnification × 100; 3B, 3C, 3F, 3E — magnification × 400

Fig. 3. Науплиусы *Oithona davisae*: A–C — особи с параллельными каудальными шипами; D–F — со скрещенными каудальными шипами; A и D — живые особи из культуры; B и E — хитиновые покровы полностью переваренных науплиусов из среднего кишечника; C и F — деформированные хитиновые покровы из нижней части кишечника. Определительные признаки: 1) стрелки указывают на пару каудальных шипов; 2) видоспецифичная форма прикрепления каудальных шипов обведена овалом. Фото: 3A и 3D — увеличение × 100; 3B, 3C, 3F , 3E — увеличение × 400

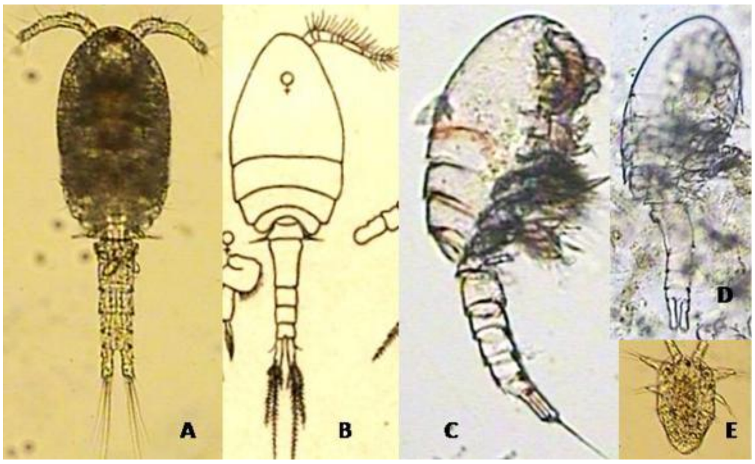

Figure 4. *Cyclopina* sp.: A — alive adult specimen from culture; B — adult specimen, drawing from [10]; C — semi-digested specimen from the midgut; digested specimen from the hindgut; D — completely digested copepod, chitin remnants (cephalothorax and abdomen); E — alive nauplius from culture. Photo: 4A–4D — magnification × 100; 4E — magnification × 400

Fig. 4. *Cyclopina* sp.: A — живая взрослая особь из культуры; B — взрослая особь, рисунок из [10]; C — полупереваренная особь из среднего отдела кишечника; переваренная особь из нижнего отдела кишечника; D — остатки хитинового покрова (цефалоторакса и абдомена) копеподита; E — живой науплиус из культуры. Фото: 4A–4D — увеличение × 100; 4E — увеличение × 400

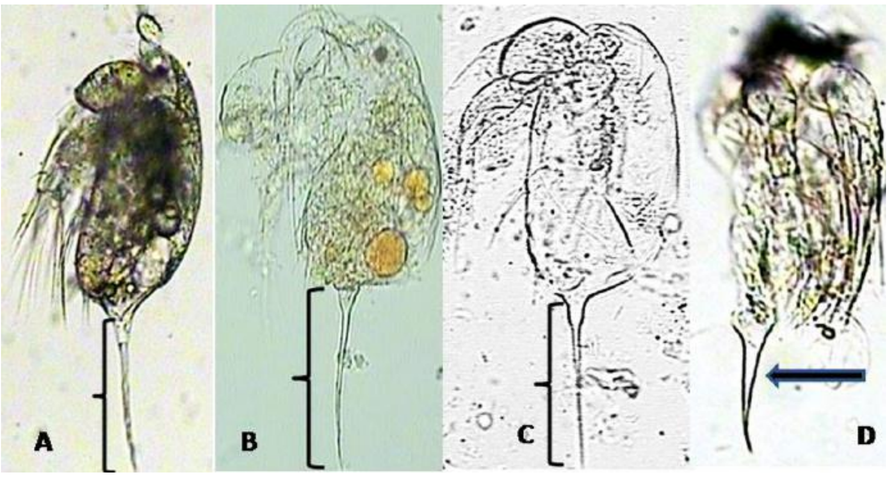

Figure 5. Naupllii of *Longipedia* sp.: A — alive specimen from culture; B — specimen from larvae fish midgut with undigested lipid drops with coloured lipid-soluble pigments; C — chitin exoskeleton of completely digested items from the hindgut; D — digested remains of Cladocera (the black spot at the top — remains of the faceted eye). Braces point at caudal process of *Longipedia* sp. nauplii. The shaded arrow point to caudal process on Cladocera carapace. Photo: 5A–5D — magnification × 100

Fig. 5. Науплиусы гарпактикоидных копепод *Longipedia* sp.: A — живая особь из культуры; B — особь из среднего отдела кишечника личинки рыбы с непереваренными вакуолями жира с жирорастворимыми пигментами; C — хитиновый покров полностью переваренной особи из заднего отдела кишечника; D — переваренные остатки Cladocera (черное пятно вверху — остатки фасеточного глаза). Фигурные скобки обозначают каудальный вырост науплиусов *Longipedia* sp. Закрашенная стрелка — каудальный вырост панциря Cladocera. Фото: 5A–5D — увеличение × 100

(fig. 1A). This specific feature (fig. 1F) starting with late copepodite stages of development of this species is the most important one and remains whatever degree of digestion in larvae and juvenile fish guts in all cases:

- in practically intact copepod is found in the foregut (fig. 1B–1C);
- in semi-digested copepod from the midgut (fig. 1C);
- after significant deformation of chitin exoskeleton when cephalothorax of *O. davisae* is found separately from abdomen, mainly in the gut (fig. 1D);
- when cephalothorax is fully deformed, compressed and twisted (fig. 1E).

Specific features of *Acartia* spp. and *O. davisae* nauplii. Both nauplii *Acartia* spp. (fig. 2) and nauplii of *O. davisae* (fig. 3) dominate in the plankton of the Black Sea coastal waters during the warm season [1, 13], are common food items of the fish at early stages of development, and it is important to distinguish from one another in the guts of fish.

Specific features of *Acartia* spp. nauplii (120–240 μm) (fig. 2) are symmetrical rows of small ventral setae and the caudal armature represented by 4 short spines and 2 long crossed setae observed at the stage of metanauplius [11, 12]. However, the caudal armature details disappear in digested *Acartia* nauplii. The main distinct specific features remained in completely digested deformed *Acartia* spp. nauplii (fig. 2D–2F) are symmetrically located rows of ventral setae in the posterior part of the body (fig. 2A–2E).

Nauplii of *O. davisae* (70–160 μm) lack similar symmetrically located rows of rows of ventral setae but their distinct features from the stage of metanauplius (85–160 μm) is their caudal armature presented by 2 pairs of caudal setae and 1 pair of long and strong caudal spines [14] attached to the small rectangular protrusion (fig. 3A–3F).

Our study revealed that such identification feature as “crossed caudal processes” (fig. 2B used in identification key [11]) cannot be applied for distinction of the remnants of nauplii *Acartia* spp. from nauplii of *O. davisae* because the caudal processes of the latter can also found crossed both in alive specimens (fig. 3D) and digested ones (fig. 3F–3E). Long caudal spines and their specific attachment mode are typical for *O. davisae* nauplii remnants. Absent from other Black Sea copepods nauplii, this species-specific feature remains unchanged even in case of strong deformation and twisting of digested chitin exoskeleton (fig. 3C, 3F).

Specific features of *Oithona* and *Cyclopina* copepods. Additionally, it is often difficult to see the difference between the remains of *Oithona* (0.22–0.28 mm) and *Cyclopina* (0.20–0.24 mm) in the guts. In case of weakly digested specimens identification is beyond doubt: shorter antennules and thickened and shortened abdomen indicates *Cyclopina* genus (fig. 4A–4D).

The rear part of *Cyclopina* sp. cephalothorax of is greatly expanded in comparison with that of *Oithona* but compressing and twisting of copepods carapaces during digestion makes this characteristic useless for identification of food items. In this case only the complex of features: absence of typical for *O. davisae* rostrum on the head and abdomen morphology of these species together with proportion of cephalothorax and abdomen should be considered (fig. 1). *Cyclopina* nauplii (65–135 μm) (fig. 4E) possess rather weak caudal processes, and lack thick caudal spines compared to those of *O. davisae*.

Specific features of *Longipedia* sp. nauplii. Another common food item in fish guts — *Longipedia* sp. (Harpacticoida) (fig. 5A–5C). No problem exists in identification of alive (fig. 5A), or partially digested (fig. 5B) *Longipedia* sp. nauplii but in case of significant digestion and deformation the caudal processes of nauplii of this species can be mistakenly can be accepted for the caudal process on Cladocera carapace (fig. 5D). Digested chitin remnants of the latter can identified in most cases by a specific black pigment indicating remains of the complex compound eye of Cladocera (fig. 5D). However, if the black pigmentation of compound Cladocera eye is absent, attention should be paid to the proportions of the caudal process: it is much longer relatively to the carapace length of *Longipedia* sp. nauplii, and in Cladocera it is shorter and has a broader base.

Thus, our research allowed us to mark out the specific chitin features of the copepod specimens that are not subject to deformation under the action of digesting processes and, therefore, are key indicators to determine the digested food items.

Conclusions. Our approach allowed us to find out specific taxonomic features of the common food items of the fish larvae and juveniles (*O. davisae*, *Cyclopina* sp., *Acartia* spp., and *Longipedia* sp.) remaining in chitin residues after digestion in the guts. Application of proposed method is proposed to use for feeding analysis of the early stages of the Black Sea fish as it is helpful for assessment of qualitative and quantitative composition of consumed common prey and selectivity. It allowed us to assess the contribution of each of these prey in the diet of the fish at early stages in Sevastopol Bays [17], and could be useful for complex analysis of ichthyoplankton changes especially during the changes in zooplankton community structure, and, thereafter for prediction of viability and survival of fish generations.

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Определение некоторых массовых пищевых объектов в кишечниках личинок и молоди рыб в Чёрном море

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В последнее десятилетие в прибрежных акваториях Чёрного моря наблюдается положительный тренд среднегодовой численности личинок рыб, который совпадает с трендом численности копепод. Есть предположение, что увеличение численности личинок рыб в Чёрном море в последние годы связано с улучшением их кормовой базы в результате интродукции нового вида копепод *Oithona davisae*. Проверка этой гипотезы с помощью исследования содержимого кишечников личинок и молоди рыб осложняется отсутствием методологической базы для определения объектов питания по их остаточным фрагментам. Цель работы — предложить собственный подход к идентификации некоторых массовых видов копепод из кишечников личинок и молоди рыб по их специфическим отличительным признакам, определяемым по непереваренным хитиновым фрагментам. Массовые виды копепод прибрежья Севастополя (*Acartia tonsa*, *Oithona davisae*, *Longipedia* sp., *Cyclopina* sp.) были выделены из природных условий в лабораторные культуры, в которых получены все последовательные стадии развития каждого вида. Для нахождения отличительных специфических признаков каждого вида проведено сравнение живых копепод на последовательных стадиях развития, их линочных шкурок и копепод из кишечников рыб на разных стадиях их переваривания. Выявлены характерные для каждого вида фрагменты и детали хитинового скелета ракообразных, которые не подвергаются деформации даже в результате пищеварительных процессов. Эти видоспецифичные признаки предложено использовать при трофологическом анализе черноморских рыб на ранних стадиях развития, в частности при оценке сезонных и локальных изменений в качественном и количественном составе потреблённой пищи и избирательных способностей рыб по отношению к определённым пищевым объектам. Предложенный метод анализа особенно важен при исследованиях в периоды изменения структуры зоопланктонного сообщества, оказывающего значимое влияние на жизнеспособность и выживаемость генераций рыб.

Keywords: пищевые объекты, личинки рыб, молодь рыб, копеподы, *Oithona davisae*, Чёрное море