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Fish eggs in the diet of White Sea cod *Gadus morhua marisalbi* (Gadiformes: Gadidae): species identification using DNA-based methods

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ABSTRACT

Trophic relationships reflect the peculiarities of the functioning of communities of aquatic organisms and analysis of their structure and dynamics can be used to improve fisheries management and assess the state of marine ecosystems. Populations at the margins of the species' range have particular adaptations, including feeding habits, to extreme conditions, which makes them a convenient model object for studying the possible consequences of ecosystem changes under the influence of global factors. The cod *Gadus morhua marisalbi* Derjugin, 1920 permanently inhabits the coastal waters of the subarctic White Sea, located at the northeastern margin of the geographic range of the Atlantic cod *G. morhua* Linnaeus, 1758. This coastal cod population strongly depends on the local environmental conditions and available food resources during the summer feeding period. In addition to the main food organisms (fish, polychaetes, crustaceans), the eggs of fish spawning in shallow coastal areas in summer are also important in the diet of cod at this time. According to published data, fish eggs found in significant quantities and most often in the stomachs of cod belonged to threespine stickleback and lumpfish, but this has not been confirmed by any experimental research. Besides, the possible presence of herring eggs in the diet of cod remains unproven due to the difficulties of its visual identification. In this work, we used molecular genetic methods for species identification of fish eggs found in cod stomachs. At the first stage, the species identification of DNA isolated from eggs was done using two sets of species-specific primers: the first which we designed for the mitochondrial COI gene of stickleback, cod, and herring, and the second, published for a microsatellite locus of herring. At the second stage, the species identity of DNA extracted from fish eggs was checked using DNA barcoding. The results showed that cod stomachs contained herring and stickleback eggs, and in a number of cases we found the co-occurrence of eggs of these species. Of the 29 DNA preparations studied, fourteen were successfully amplified with microsatellite primers for herring, and thirteen amplified with primers for stickleback. Interannual differences in the occurrence of herring eggs in samples were insignificant, while the frequency of occurrence of stickleback eggs in 2018 was higher than in 2017. Our analysis did not reveal significant intra-annual differences between the observed and theoretically expected number of cases of simultaneous occurrences of herring and stickleback eggs. The high frequency of occurrence of herring and stickleback eggs in cod stomachs revealed in this study indicates the importance of these components in the diet of cod and provides new knowledge on the trophic role of cod as a key species in the White Sea ecosystem. Analysis of available data on the spatial and temporal diet patterns of coastal White Sea cod shows that fish eggs can significantly contribute to the total amount of food consumed by this species during the summer.

Key words: cod, DNA-analysis, eggs, feeding, herring, stickleback

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Икра рыб в составе пищи трески *Gadus morhua marisalbi* (Gadiformes: Gadidae) Белого моря: видовая идентификация с использованием молекулярно-генетических методов

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РЕЗЮМЕ

Трофические связи гидробионтов отражают особенности функционирования прибрежных сообществ, и анализ их структурной динамики может быть использован для улучшения менеджмента рыбного промысла и оценки состояния морских экосистем. Популяции на границах ареала вида имеют особые адаптации, в том числе пищевые, к экстремальным условиям, что делает их удобным модельным объектом для изучения возможных последствий изменения экосистем под влиянием глобальных факторов. Треска *Gadus morhua marisalbi* Derjugin, 1920 постоянно обитает в прибрежных водах субарктического Белого моря, расположенного на северо-восточной окраине ареала атлантической трески *G. morhua* Linnaeus, 1758. Структура и динамика популяции прибрежной трески сильно зависят от местных условий окружающей среды и имеющихся пищевых ресурсов в летний период нагула. Помимо основных пищевых объектов (рыб, полихет, ракообразных), в пище трески в это время существенное значение имеет икра рыб, нерестящихся летом на мелководных прибрежных участках. По литературным данным икра рыб, обнаруженная в значительном количестве и чаще всего в желудках трески, принадлежала трехиглой колюшке и пинагору, однако это не было подтверждено экспериментальными исследованиями. Кроме того, возможное присутствие икры сельди в рационе трески остается недоказанным из-за трудностей ее визуальной идентификации. В данной работе мы использовали молекулярно-генетические методы для видовой идентификации икры рыб, обнаруженной в желудках трески. На первом этапе проводили видовую идентификацию ДНК, выделенной из икры, с использованием микросателлитных праймеров и разработанных нами видоспецифичных маркеров для митохондриального гена COI. На втором этапе проверяли видовую принадлежность ДНК, выделенной из икры рыб, с помощью ДНК-штрихкодирования. Результаты показали, что в желудках трески находилась икра сельди и колюшки, а в ряде случаев обнаружено совместное присутствие икры этих видов. Из 29 изученных препаратов ДНК четырнадцать были успешно амплифицированы с помощью микросателлитных праймеров для сельди, а тринадцать – с праймерами для колюшки. Межгодовые различия встречаемости икры сельди в пробах были незначительными, а частота встречаемости икры колюшки в 2018 г. была выше, чем в 2017 г. Проведенный анализ не выявил существенных внутригодовых различий между наблюдаемым и теоретически ожидаемым количеством случаев одновременного присутствия в желудках трески икры сельди и колюшки. Выявленная в работе высокая частота встречаемости икры сельди и колюшки в желудках трески свидетельствует о важности этих компонентов в рационе трески. Полученные сведения расширяют наши представления о трофической роли трески как ключевого вида в экосистеме Белого моря. Анализ имеющихся данных о пространственном и временном характере питания прибрежной беломорской трески показывает, что икра рыб может вносить существенный вклад в общее количество пищи, потребляемой этим видом в течение лета.

Ключевые слова: треска, ДНК-анализ, икра, питание, сельдь, колюшка

INTRODUCTION

The study of trophic webs in marine ecosystems is crucial for understanding the functioning of communities of hydrobionts. Trophic interactions of fish species are based on the availability and abundance of various prey items, and a major challenge in feeding ecology is to answer how diet composition of fish varies over time and space scales and what are the causes of these changes. Possible sources of considerable

variations in diet of fish include the changes in habitats due to anthropogenic impact or continued effects of global warming, marked changes in the abundance of key prey species as the result of commercial exploitation or long-term population dynamics, the introduction of new invasive species into the ecosystem, and others (Gjøsæter et al. 2009; Johannesen et al. 2012; Kortsch et al. 2015; Eriksen et al. 2017). Understanding the mechanisms of such changes is important for creating a basis for improving the management of fisheries and marine ecosystems, as well as for developing measures for the rational use of fish stocks. The food preferences of fish from different populations can vary significantly, which reflects the status of the food base and the peculiarities of the feeding strategy of this species in local habitat conditions. Changes in the composition of fish diet can be particularly significant in populations living at the margins of a species' range. Studying such populations can provide valuable information about the possible changes in fish trophic interactions and population processes during significant shifts in ecosystems.

The Atlantic cod *Gadus morhua* Linnaeus, 1758 is distributed over a large geographic area in the North Atlantic and adjacent Arctic (Mecklenburg et al. 2018). The main stocks of cod (Northeastern Arctic cod, Norwegian coastal cod, and White Sea cod), inhabiting Arctic and subarctic waters of Northeast Atlantic, differ in distribution, migration patterns, life-history traits and feeding ecology (Brander 1994; Novikov 1995; Nordeide and Båmstedt 1998; Michalsen et al. 2008). In Arctic and coastal sub-Arctic areas, cod is one of the main top predators feeding on fish and a large array of other prey taxa. The most important fish that cod feeds on in these regions are capelin *Mallotus villosus* (Müller, 1776), herring *Clupea harengus* Linnaeus, 1758, haddock *Melanogrammus aeglefinu*s (Linnaeus, 1758), Norway pout *Trisopterus esmarkii* (Nilsson, 1855) and some others (Mehl and Sunnanå 1991; Dolgov et al. 2007; Michalsen et al. 2008; Durant et al. 2014). However, coastal areas of Norway and the White Sea (Russia) are assumed to be less productive than the Barents Sea basin and relatively stationary stocks of cod, inhabiting the fjord systems and inlets with very different environmental conditions, might strongly depend on the local food resources (Sakshaug et al. 1994; Berger 2007). It was shown that the diet composition of coastal fjord cod changed significantly from south to north of Norway and even within the same fjord depending on the distribution and abundance of benthic invertebrates and fish inhabiting it (Hop et al. 1992; Kanapathippillai et al. 1994; Enoksen and Reiss 2017). A convenient model object for studying changes in the diet of coastal cod in marginal populations is, in particular, the cod population of the White Sea, a region located on the eastern border of the species' range.

Cod *G. morhua marisalbi* Derjugin, 1920, one of the subspecies of the Atlantic cod (Stroganov 2015), is a typical representative of the White Sea ichthyofauna, which includes about 60 species (Rass 1995). The coastal waters of Kandalaksha Bay and the area surrounding the Solovetsky Islands have the highest recorded abundance of cod, while no cod have been reported from Dvina and Mezen Bays, and most of Onega Bay (Altukhov et al. 1958). White Sea cod is a demersal fish that permanently inhabits coastal areas and inlets, migrating over short distances along the shore (Sonina 1957). The population of this species plays an important role in marine coastal food webs and can have large impacts on other trophic levels. It does not form large aggregations or schools in its feeding grounds and is not of great importance to the White Sea fisheries.

The existing publications cover various aspects of the biology of this fish in the White Sea, including the composition of the cod diet and its seasonal and local variability (Yevropeytzeva 1937; Izvekova 1964; Kudersky 1966; Karpov et al. 1984; Novikov 1995). Most of the published data on White Sea cod focuses on different regions of Kandalaksha Bay, where this species has its primary feeding grounds in numerous inlets along the coastline and in the coastal waters at the head of the bay (Altukhov et al. 1958; Mukhomediyarov 1963a). Cod living in the White Sea is an omnivorous species that preys upon a broad range of organisms (Yevropeytzeva 1937; Sonina 1957; Kudersky 1966, etc.). For instance, Izvekova (1964) identified 79 species of food organisms in the summer diet of cod caught in the Velikaya Salma Strait area (Kandalaksha Bay). Cod feeds primarily on fish, crustaceans, and polychaetes. The fish species consumed by cod are threespine stickleback *Gasterosteus aculeatus* Linnaeus, 1758, herring *Clupea pallasii marisalbi* Berg, 1923, rock gunnel *Pholis gunnellus* (Linnaeus, 1758), slender eelblenny *Lumpenus fabricii* Reinhardt, 1836, shorthorn sculpin *Myoxocephalus scorpius* (Linnaeus, 1758) and a few others.

In summer, the cod diet also includes the eggs of some fish species that spawn during that time of year. For instance, in June to July, threespine stickleback spawns in thickets of eelgrass *Zostera marina* Linnaeus, 1753 and fucoid seaweeds in the shallow areas of inlets of Kandalaksha Bay (Altukhov et al. 1958; Mukhomediyarov 1963a, 1966; Mikodina 1978). The female stickleback lay eggs in nests, which are guarded by males. The fast-growing summer-spawning herring ("ivanovskaya" ecological form) also spawns in June in the upper sublittoral zone of Kandalaksha Bay at depths up to 5 m (Altukhov et al. 1958; Galkina 1962; Soin 1963). Herring deposits its eggs on vegetation, mostly on *Zostera*, *Fucus*, *Laminaria*, and occasionally on rocks (Soin 1963; Dushkina et al. 1981). The reproductive season of another species, lumpfish *Cyclopterus lumpus* Linnaeus, 1758, begins in late May and continues until mid-July (Altukhov 1979; Parukhina 2005). The peak of spawning of this species occurs in June in the coastal shallow-water zone (Prozorov 1948; Mochek 1973; Mikulin 1981a). The eggs are benthic and adhesive, the females lay them in clusters on rocky-sandy substrates or among seaweeds (Altukhov et al. 1958). The eggs of these three species have previously been found in the diet of cod caught in different areas of Kandalaksha Bay in June and July (Sonina 1957; Kudersky and Rusanova 1963; Parukhina 2005; Ershov 2010; Bakhvalova et al. 2016). The frequency of occurrence of stickleback eggs in the cod stomachs was significantly higher than that of lumpfish eggs. The herring eggs in cod food were recorded only once (Yevropeytzeva 1937).

It must be emphasized that, to date, there is no documented evidence of the simultaneous presence of eggs from different fish species in the cod diet, despite the fact that they spawn during relatively similar periods. This is surprising, because the spawning of herring and stickleback occurs mostly over the same substrate, in the thickets of *Zostera* and *Fucus*, at shallow depths, and lumpfish also deposit their eggs in coastal shallow-water areas among seaweeds and rocks. It can be proposed as an explanation that accurate visual identification of fish species from the eggs found in the cod stomachs was often difficult or impossible because the eggs of fish species that spawn during this period are similar in size. Some authors (Yevropeytzeva 1937; Parukhina 2005) described the fish eggs found in the cod stomachs as belonging to an unidentified species, which can be regarded as indirect evidence in favor of this assumption. According to published data, there is wide variation in egg size among these three species. For instance, the diameter of fertilized eggs from fast-growing summer-spawning herring in Chupa and Palkina Inlets of Kandalaksha Bay ranged from 1.21 to 1.46 mm in different individuals (Yastrebkov 1969) and was directly proportional to the size/age of the females. The eggs of stickleback from Chupa Inlet and the Velikaya Salma Strait were slightly larger (1.6–1.8 mm), compared to those of herring (Mukhomediyarov 1966; Mikodina 1978). The largest eggs were those of lumpfish, with an average diameter ranging from 1.6 to 2.7 mm (typically 2.1–2.3 mm) in different females (Prozorov 1948; Soin and Mikulin 1974; Altukhov 1979; Mikulin 1981a). These data show a partial overlap in the variability of egg sizes between stickleback and lumpfish, which is further complicated by the observation that at the onset of the lumpfish spawning period, in June, the littoral zone is approached by small-sized females whose eggs are smaller in diameter than in later-spawning larger individuals (Mochek 1973; Mikulin 1981a). Consequently, small inter-species differences and high intra-population variability in egg diameter observed in all three species impose serious limitations on the utility of this character as a criterion for visual identification of the "owner" of the eggs found in the cod stomachs. Another characteristic, egg color, poses similar challenges for accurate differentiation of herring, stickleback, and lumpfish eggs, because the digestive enzymes in the cod stomach can alter the egg color and all three species have eggs with a similar yellowish tint (Soin 1963, 1980; Mikulin et al. 1978; Mikulin 1981b; Zyuganov 1991).

To our knowledge, fish eggs have not been found in the diet of coastal cod in Norway and nearshore the Kola Peninsula (Russia), despite the fact that the food composition of Atlantic cod in these regions is relatively well studied (Klementsen 1982; Hop et al. 1992; Kanapathippillai et al. 1994; Michalsen et al. 2008; Enoksen and Reiss 2017). Species identification of fish eggs found in the stomachs of coastal cod, living in the northeastern part of the species' range (subarctic White Sea), is therefore of particular interest. Chupa Inlet of the Kandalaksha Bay is known to be a site for mass spawning of stickleback, herring, and lumpfish (Mukhomediyarov 1963b, 1966; Altukhov 1979). Previous studies conducted during the summer months at the mouth of Chupa Inlet

have shown that the cod diet includes stickleback and lumpfish eggs (Ershov 2010; Bakhvalova et al. 2016), with the egg species identified visually by the authors. However, herring eggs have not been reported for the cod diet, despite the presence of extensive spawning grounds of herring in this and other areas of Kandalaksha Bay. Considering the difficulties mentioned earlier in identifying the species of fish eggs based on morphological characters, we chose to address this problem using DNA-based identification methods. The first objective of this study was to develop molecular genetic tools to accurately identify fish eggs consumed by White Sea cod. The second aim was to answer the question of whether or not herring eggs are part of the diet of cod in its typical feeding grounds in Kandalaksha Bay of the White Sea. Third, we tried to obtain preliminary estimates of the contribution of fish eggs to the summer diet of coastal White Sea cod.

MATERIAL AND METHODS

Species identification of the eggs extracted from cod stomachs was performed by molecular-biological methods. Our approach was two-fold: (1) species identification of DNA isolated from eggs using species-specific primers, which we designed, and (2) verification of the species identity of the extracted DNA by COI-based DNA barcoding (Ivanova et al. 2007). Since the cod stomach contains a mixture of DNA from a variety of prey items, the primary challenge in performing DNA barcoding is to extract DNA fragments that incorporate the necessary regions in a pure form. To address this problem, we designed the COI-based primers in such a way as to ensure that the resulting sequence contains the region used in fish species identification (Ivanova et al. 2007). This allowed for additional verification that strengthened the accuracy of species identification of DNA made using species-specific primers.

Sample preparation and DNA extraction

Material for the study was collected at the mouth of Chupa Inlet (Kandalaksha Bay, White Sea) in areas adjacent to the White Sea Biological Station of the Zoological Institute of the Russian Academy of Sciences: Seldyanaya Inlet, Kruglaya Inlet, and Kartesh Cape (Fig. 1). Cod were captured during the period from mid-June to mid-July 2017 and 2018 in coastal shallow-water areas at depths ranging from 2 to 5 m, using gillnets with mesh sizes of 30–40 mm. The stomach contents of the captured fish (total

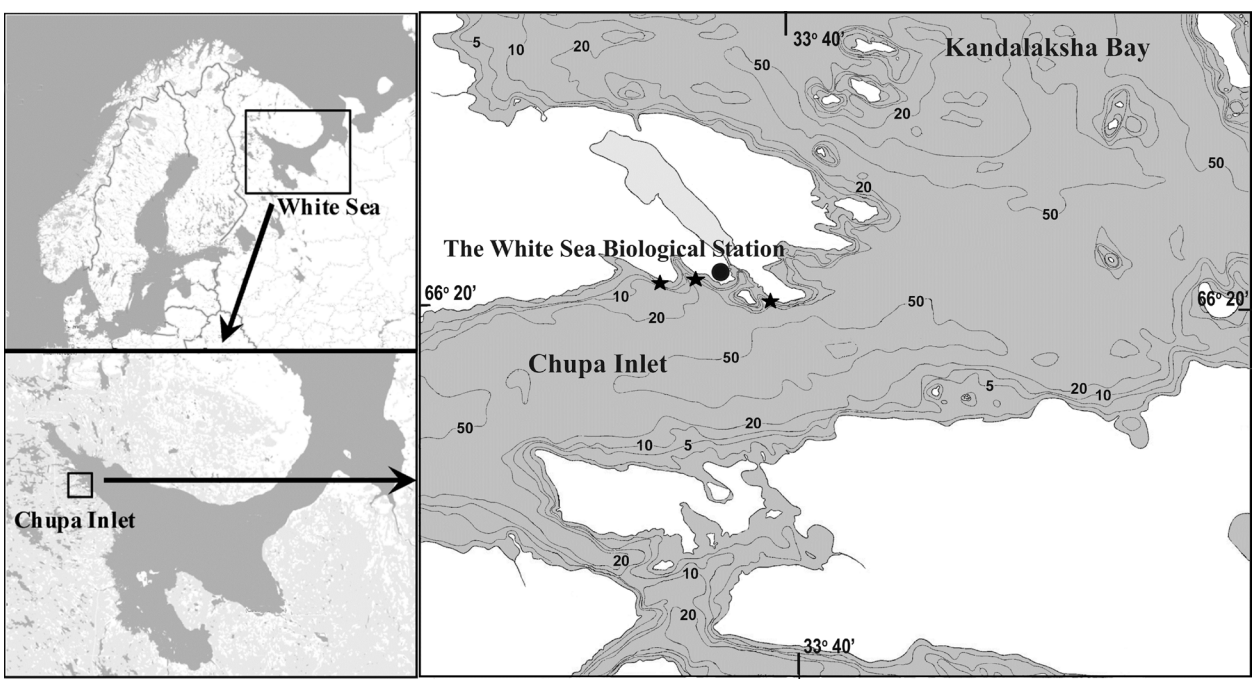

Fig. 1. Map showing the area of investigation. Asterisks indicate locations of sampling. The numbers indicate depths in meters.

Fig. 2. Fish eggs from the stomach of a cod specimen.

body length of 26.3 to 43.2 cm) were analyzed for the qualitative composition of food. Cod individuals that contained eggs in their stomachs (Fig. 2) were selected from the general sample for further analysis (n = 46). Stomachs of cod containing both eggs and partially digested mature female threespine sticklebacks were omitted from further analysis to avoid the inclusion of mature eggs from these individuals in the samples. None of the cod stomachs contained adult herring with mature eggs in their gonads. The stomach contents of selected fish were analyzed according to the relative importance in wet weight for eggs and prey species.

Egg samples from the stomachs of 46 cod individuals, each weighing 2–3 g, were mechanically cleaned using a scalpel to remove mucus and remnants of digested food, and were fixed in 3 mL of 96% ethanol. The fixative in the samples was changed twice within one day after the initial fixation, using 70% ethanol. DNA was extracted from the egg using a modified phenol-chloroform method. Egg samples were placed in sterile microcentrifuge tubes containing 700 µL of lysis buffer with proteinase K (7 µL of 1M Tris-HCl (pH 8.0) + 135 µL of 0.5M EDTA (pH 8.0) + 531 µL of PCR grade water + 17 µL of 20% SDS + 10 µL of proteinase K solution (10 mg/mL) for each 700 µL of the buffer), and then the samples were mechanically disrupted with a sterile pestle. After mixing, the tubes were placed in a thermostat for 16 hours at 55°C. An equal volume (700 µL) of a phenol-chloroform-isoamyl alcohol mixture (25:24:1) was then added to the digest and the resulting solution was mixed and centrifuged at 4°C for 10 min at the maximum speed (20800 g). A total of 1000 µL of the supernatant was transferred to a clean tube, centrifuged again, and then the supernatant was transferred to a new tube, while taking care not to touch the phase boundary. An equal volume of a phenol-chloroform-isoamyl alcohol mixture (25:24:1) was added again to the supernatant, and then the above-described procedure was repeated. After that, an equal volume of a chloroform-isoamyl alcohol mixture (24:1) was added to the resulting supernatant, the solution was mixed thoroughly, centrifuged, and the supernatant was transferred to a clean tube. For DNA precipitation, 500 µL of 96% cold EtOH and 1 µL of glycogen solution (5 mg/µL) were added to the supernatant, mixed, and incubated at −20°C for 20 min. The tubes were then centrifuged at 4°C for 10 min at the maximum speed, and the supernatant was removed using a mechanical pipette. The precipitate was washed again with 500 µL of 70% ethanol for 3–5 min and centrifuged at 4°C for 5 min at the maximum speed. After removing the supernatant, the DNA precipitate was air-dried for 10 min in a thermostat at 37°C; 30 µL of PCR grade water was added, and it was left for one day at +4°C for complete dissolution. The quality of extracted DNA was assessed through amplification with modified universal primers (see below). A total of 29 preparations containing the amplifying DNA were used for further analysis. DNA could not be extracted from approximately one third of collected egg samples (17 out of 46), probably because of the small amount of mitochondrial DNA present in the semi-digested eggs.

Species identification of extracted DNA using species-specific primers

Threespine stickleback and herring were chosen as test “host” species for the eggs. Samples were not assessed for the presence of lumpfish DNA, because, at the time of the study, GenBank NCBI contained no information on the complete mitochondrial genome of *C. lumpus*. For stickleback and herring, species-specific primers were designed based on the COI gene sequence, using data from the complete mitochondrial genomes of these species obtained from GenBank NCBI. For stickleback, data were derived from the mi-

Table 1. Primers used in COI and microsatellite DNA amplification.

Name	Primer sequence 5–3′	References
Gadus_FR	TCTCTGCTATGCTCTTGCCCC	This study
Gasterosteus_FR	TTTACTTCTACCATTGGCCCC	This study
Clupea pallasii _FR	TGTCTCCTACCTCTCACTCCC	This study
Cpa110_FR	CTGACAACCCTCGACATACAT	Semenova et al. 2013
Cpa110_RV	ACAATTTGCACTGGTTTGTAGTAG	Semenova et al. 2013
Fish_FR	TTCTCRACYAATCACAAAGAYATTGG	Ivanova et al. 2007
Fish_RV	CYTCVGGRTGBCCRAARAATCARAA	Ivanova et al. 2007

Note. Designations are given in accordance with IUPAC recommendations.

togenomes of *Gasterosteus wheatlandi* Putnam, 1867 (NC_011570) and *G. aculeatus* (AP002944). The location of the barcode region in the mitochondrial genome was determined using the COI sequence of *G. aculeatus* (KX145452). Data on herring were taken from the mitochondrial genomes of *Clupea pallasii* Valenciennes, 1847 (NC_009578) and *Clupea harengus* (NC_009577). The COI sequence of *C. pallasii* (JF693633) was used to identify the location of the barcode region in the genome. In addition, species-specific primers for cod were designed to detect possible contamination of prey egg samples by DNA of the predator. The primers were made based on the data from three complete mitochondrial genomes of *Gadus morhua* (X99772, HG514359, AM489716). The location of the barcode region in the genome was determined using the corresponding COI sequence of *G. morhua* (KC015374). Species-specific primers were developed using BioEdit 7.2.5 (identification of species-specific regions, https://bioedit.software.informer.com/7.2/) and Primer Premier 5.0 (design and analysis of primers, https://primer-premier-5.software.informer.com/). Forward primers (Table 1) were species-specific for stickleback, cod, and herring and were complementary to the unique sequences located approximately 450 bp in the 5' direction from the annealing site of the forward barcoding primer proposed by Ivanova et al. (2007). In all cases, the reverse primer Fish_RV corresponded to that proposed by Ivanova et al. (2007) for fish DNA barcoding. The region used for fish DNA barcoding was therefore included in the sequence amplified using species-specific primers designed for this study.

During the course of the study, species-specific primers for herring and modified (see below) universal primers were found to work only when applied to freshly extracted DNA from herring eggs. After 4–5 months, herring DNA ceased to amplify with both species-specific and modified universal primers. We believe that this was probably caused by the small amount of mitochondrial DNA present in the semi-digested eggs and its rapid degradation. Therefore, the subsequent identification of herring DNA in the egg samples extracted from cod stomachs was conducted using microsatellite primers developed for DNA of White Sea herring by Semenova et al. (2013). In the present study, we used the microsatellite locus *Cpa110*, in which White Sea herring was shown to have 5 alleles 138–162 bp in length. The presence of the amplicon in the corresponding length range during electrophoresis on a dense (2%) agarose gel was considered as the positive signal, indicating the presence of herring DNA. Species-specific primers were synthesized by Biobeagle (Russia, https://biobeagle.com).

The estimated annealing temperature for the designed primers was calculated using the OligoAnalyzer 3.1 (https://softadvice.informer.com/Oligo_Analyzer_3.1_64bit.html) and OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html) programs. The selection of the optimal annealing temperature (Table 2) and verification of amplification conditions (reagent ratios) were carried out through amplification in a temperature gradient in an Applied Biosystems ProFlex thermal cycler.

The validation of species specificity of the primers designed by authors was conducted using DNA extracted from the muscle tissue of the respective fish species with a "DNA-Extran-2" kit following the manufacturer's guidelines (Syntol Research & Production Company, Russia, https://www.syntol.ru). Ten samples of cod DNA, ten samples of stickleback DNA and 6 samples of herring DNA were used for validation.

Table 2. Optimal annealing temperature for designed primers and the length of amplified fragments.

Primer 1	Primer 2	T, °C	Fragment length, bp
Gadus_FR	Fish_RV	64	1162
Gasterosteus_FR	Fish_RV	60	1155
Clupea pallasii _FR	Fish_RV	64	1158
Cpa110_FR	Cpa110_RV	52	138–162
Fish_FR	Fish_RV	52	705

PCR amplification of species-specific primers was performed using ScreenMix kits (Evrogen JSC, Russia, https://evrogen.ru/), which include all necessary components for PCR and agarose gel electrophoresis.

The reaction mixture (25 μL) for the primers designed for the mitochondrial COI gene sequence contained 5 μL of ScreenMix mixture, 1 μL each of the solutions of the species-specific primer and Fish_RV primer (at 10 pM/μL), 100 ng of total DNA, and 18 μL of PCR grade water. Amplification was performed in an Applied Biosystems ProFlex thermal cycler under the following cycling conditions: denaturation at 94°C for 2 min, followed by 35 cycles, each consisting of DNA template denaturation at 94°C for 30 s, primer annealing at the specified temperature (Table 2) for 40 s, and new strand synthesis at 72°C for 75 s, with a final extension of 10 min at 72°C.

DNA was prepared for sequencing using a similar protocol, but the volume of the incubation mixture was doubled to obtain the required amount of purified amplicon.

The reaction mixture (20 μL) for microsatellite primers consisted of 4 μL of ScreenMix mixture, 3 μL each of Cpa110_FR and Cpa110_RV primer solutions (at 10 pM/μL), 100 ng of total DNA, and 10 μL of PCR grade water. Amplification was performed in an Applied Biosystems ProFlex thermal cycler under the conditions described by Semenova et al. (2013).

The amplification products were separated by electrophoresis on 1.5% and 2.0% agarose gels (1.5–2.0 g agarose + 100 mL 1X TAE + 4 μL ethidium bromide solution), in 1X TAE buffer at 100V and photographed under UV light. The DNA GeneRuler 100 bp Plus molecular weight marker (Thermo FS) was used as a fragment length marker. The mobility of the yellow dye in the ScreenMix reaction mixture corresponded to fragment lengths of 20–30 bp.

Barcode-based validation for species identification of DNA extracted from eggs

Ivanova et al. (2007) proposed using the sequence of the COI mitochondrial gene for species identification of fish and developed universal degenerate primers for fish barcoding (FF2d and FF1d). To increase the specificity of these primers for our specific purposes, we made small modifications based on the sequence analysis of the complete mitochondrial genomes of herring, stickleback, and cod available in GenBank NCBI. The resulting modified primers Fish_FR and Fish_RV (Table 1) are analogous to universal primers FF2d and FF1d (Ivanova et al. 2007), respectively. The annealing temperature (Table 2) and amplification conditions for the modified universal primers were optimized using the same procedure as for the species-specific primers (see above). The modified primers were also synthesized by Biobeagle.

PCR amplification of modified universal primers was performed using ScreenMix kits (see above). The reaction mixture (25 μL) contained 5 μL of ScreenMix mixture, 1 μL each of Fish_FR and Fish_RV solutions (10 pM/μL), 80 ng of total DNA, and 18 μL of PCR grade water. Amplification was carried out in an Applied Biosystems ProFlex thermal cycler under the following cycling conditions: denaturation at 94°C for 2 min, followed by 40 cycles of DNA template denaturation at 94°C for 30 s, primer annealing at 52°C for 40 s, and new strand synthesis at 72°C for 75 s, with a final extension of 10 min at 72°C. The amplification products were separated by electrophoresis on a 1.5% agarose gel (1.5 g agarose + 100 mL 1X TAE + 4 μL ethidium bromide solution) in 1X TAE buffer at 100 V and photographed under UV light. Fragment lengths were estimated using the GeneRuler 100 bp Plus DNA molecular weight marker.

To obtain sequences purified from extraneous DNA, DNA samples extracted from the eggs were initially amplified with species-specific primers. This procedure proved to be impossible in herring due to the rapid degradation of its mitochondrial DNA. DNA sequences from stickleback and cod limited by species-specific primers were then amplified using modified universal primers (Fish_FR and Fish_RV; 4 tubes, 25 μL each, totaling 100 μL per sample) and separated from side products by electrophoresis on a 1.5% agarose gel. PCR products were purified us-

ing a KR-011 DNA purification kit by Omnix LLC (Russia, https://omnix.ru) following the manufacturer's guidelines. The extracted DNA fragments were Sanger-sequenced by Biobeagle (Russia, https://biobeagle.com) using a MegaBACE 1000 automatic capillary DNA Analysis System.

Statistical analysis

The statistical analysis of the results was conducted using standard statistical methods. An exact two-tailed test for comparing the empirical frequency with a specified frequency for the binomial distribution, as described by Lloyd and Lederman (1989), was used to compare the expected and observed co-occurrence frequencies of eggs from different fish species. Deviations corresponding to the upper and lower limits of the exact 95% interval were calculated for the frequencies of egg occurrence for different fish species (Lloyd and Lederman 1989). Identities (*I*) of the barcode sequences, calculated as 1 – p-distance, and the number of base pairs differing between sequences were computed using BioEdit 7.2.5.

RESULTS

Brief information on stomach content of cod

The food composition of 46 cod individuals selected for the study included eggs, polychaetes, fish, and in half of the examined stomachs the eggs were the only food item. In the majority of the examined stomachs (90%), fish eggs dominated by weight among food items. The relative weight of fish eggs in the content of stomachs was 85%, and in absolute terms, the weight of fish eggs eaten by some cod individuals reached 28 g. Polychaetes were represented by two species, *Alitta virens* (M. Sars, 1835) and *Nereis pelagica* Linnaeus, 1758, which accounted only for 4% by weight. The number of recorded prey fish species was small, with only 3 species found in cod stomachs (rock gunnel, slender eelblenny, lesser sandeel *Ammodytes marinus* Raitt, 1934). The proportion of fish in the studied stomach content was small and amounted to 11% by weight.

Validation of primer specificity

Three groups of DNA samples from fish tissues were used to validate species-specific primers. Each group included 26 samples, consisting each of 10 total DNA samples from cod and stickleback and 6 samples from herring. DNA samples from each group were amplified with a pair of species-specific primers (Gadus_FR – Fish_RV, Gasterosteus_FR – Fish_RV, Clupea pallasii_FR – Fish_RV). The amplification results are shown in Fig. 3.

During the amplification of cod DNA preparations using the Gadus_FR / Fish_RV primers, the band was observed at the expected position (approximately 1162 bp). In some cases, faint bands were also present at around 280 and 3500 bp. Stickleback and herring exhibited a varying degree of nonspecific amplification, but there was no pronounced band at 1162 bp. In herring DNA preparations, a distinct amplicon was present at 1500 bp and there were faint bands at 550 bp and 750 bp. The PCR products generated from stickleback DNA extracts showed several weak bands characteristic only of this species.

As a result of the amplification of stickleback DNA extracts with the Gasterosteus_FR / Fish_RV primer pair, a strong PCR product was generated at the expected position of around 1155 bp and additional faint bands specific to this species were also present. The PCR product generated from the cod preparations had a read length of 1500 bp. There were also several additional, weak species-specific bands, including one approximately at 900 bp. Herring preparations showed an extremely faint amplicon at 1450 bp, and in some cases, a barely noticeable band at 450 bp. Overall, the amplification patterns were species-specific in all species.

During the amplification of herring DNA preparations with the Clupea pallasii_FR / Fish_RV primers, a distinct PCR product was generated at the expected position (approximately 1158 bp). Cod DNA preparations had three faint amplicons in the range of 750–1000 bp. Stickleback preparations showed no apparent specific or non-specific amplification. Thus, the results of DNA amplification with the Clupea pallasii_FR / Fish_RV primers also allow reliable discrimination of all species.

The amplification reaction with the Cpa110_FR / Cpa110_RV primers was successful only for DNA preparations from herring muscle tissue. The band corresponding to the microsatellite locus *Cpa110* was located at the expected position (approximately 150 bp). No amplification was detected for the cod and stickleback DNA; in some cases, a faint nonspecific band was observed at around 400 bp.

It can therefore be concluded that the DNA fragments (bp) amplified using primers specific to each species had the expected length. The amplification

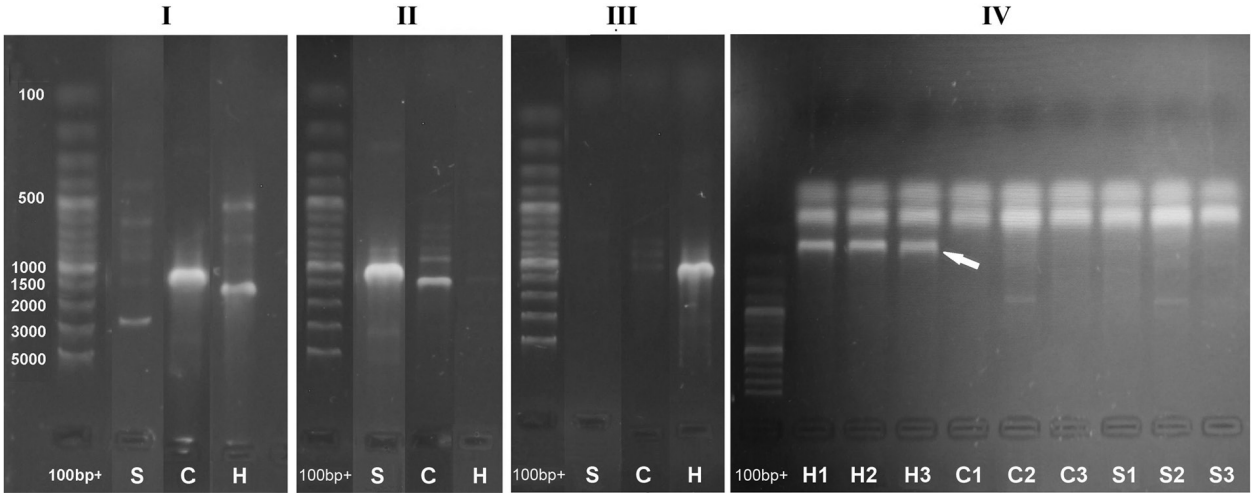

Fig. 3. Validation results for species-specific primers. Figs I, II, and III are reconstructions; each shows a fragment of an electrophoregram with PCR products, which were generated with a pair of species-specific primers for DNA preparations of stickleback, cod, and herring (see the text for explanations). *Abbreviations:* Gadus_FR / Fish_RV primers (I), Gasterosteus_FR / Fish_RV primers (II), Clupea pallasii_FR / Fish_RV primers (III), Cpa110_FR / Cpa110_RV primers (IV); stickleback (S), cod (C), herring (H); DNA GeneRuler 100 bp plus molecular weight marker (100bp+). The arrow indicates the position of the band corresponding to the amplification results with the Cpa110_FR / Cpa110_RV primers.

with different pairs of species-specific primers provides reliable discrimination for all studied species.

Species identification of extracted DNA using species-specific primers

At the next stage, DNA of the eggs taken from the stomachs of 29 cod individuals was amplified using three pairs of species-specific primers designed by the authors. The results of identification of egg DNA amplified using species-specific primers are summarized in Table 3.

DNA amplification was successful on 8 samples amplified with the Gadus_FR / Fish_RV primers (Table 3). The observed bands matched those of cod (approximately 1162 bp), and no additional bands were present. As noted earlier, the other two species, stickleback and herring, did not exhibit any distinct bands of this length. Eight out of 29 DNA preparations of eggs were therefore significantly contaminated with DNA from the digestive tissues of cod itself.

Thirteen DNA extracts were successfully amplified using stickleback primers (Gasterosteus_FR / Fish_RV) (Table 3). The band was located at the expected position (approximately 1155 bp). Additional bands, when visible, corresponded to those obtained by amplification of DNA preparations from stickleback soft tissues. There was no evidence of nonspecific amplification of these primers with DNA from other species. If cod DNA was present, bands at 1500 bp and around 900 bp should have been visible, but they were not detected during electrophoresis. Herring showed no nonspecific amplification with the Gasterosteus_FR / Fish_RV primers.

DNA preparations from herring eggs, unlike those from soft tissues, did not amplify with herring-specific primers (Clupea pallasii_FR / Fish_RV). No evidence of specific or nonspecific amplification was detected.

Fourteen DNA preparations were successfully amplified with microsatellite primers (Table 3). In all cases, a band of approximately 150 bp was clearly visible, which corresponded to the expected fragment length for the selected microsatellite locus.

DNA extracts from four samples (Table 3) failed to amplify with any of the three pairs of species-specific primers, although these samples had previously been shown to contain DNA that was successfully amplified with universal barcoding primers. Since cod consumes not only herring and stickleback eggs but also those of lumpfish, it can be hypothesized that these samples contained lumpfish DNA.

The results of the analysis showed that the eggs from cod stomachs belong at least to two fish species, stickleback and herring, and that in some cases cod stomachs contained the eggs of both these species simultaneously. It should also be noted that the level of

Table 3. DNA identification results for the examined samples using species-specific primers. A plus sign indicates the presence of DNA in the samples.

Sample No.	Catch date	DNA		
		Cod	Stickleback	Herring
3	26.06.2017	–	–	+
18	29.06.2017	–	–	+
19	29.06.2017	–	+	–
35	29.06.2017	–	–	–
36	29.06.2017	–	–	+
50	01.07.2017	–	–	+
52	01.07.2017	+	+	–
9	02.07.2018	–	+	+
10	02.07.2018	+	+	–
29	04.07.2018	–	–	–
59	06.07.2018	–	–	+
66	06.07.2018	+	+	–
69	07.07.2018	+	+	–
70	07.07.2018	+	+	–
83	07.07.2018	–	+	+
85	07.07.2018	+	+	–
107	08.07.2017	–	–	+
111	08.07.2017	–	–	+
168	09.07.2017	–	–	+
172	09.07.2017	–	–	+
173	09.07.2017	–	–	–
106	09.07.2018	–	+	–
122	10.07.2018	+	+	–
124	10.07.2018	–	–	+
125	10.07.2018	–	–	–
157	11.07.2018	–	–	+
158	11.07.2018	+	+	–
160	14.07.2018	–	+	–
179	15.07.2018	–	–	+

nonspecific DNA amplification in soft tissue samples was significantly higher than that observed for DNA amplification of egg samples.

Barcode-based selective validation for species identification of DNA extracted from eggs

The fragments of mitochondrial DNA from stickleback and cod, obtained using species-specific primers, contained the barcode regions of the COI gene, which provided an additional level of verification for the accuracy of species identification.

Samples Nos. 66, 70, 158 of cod DNA preparations and samples Nos. 9, 66, 158, 160 of stickleback DNA preparations were chosen for selective validation. All selected DNA preparations (except sample No. 160) contained a mixture of DNA from two fish species (Table 3). DNA fragments from cod and stickleback in these samples were purified from extraneous DNA through amplification with species-specific primers. Sample No. 160 contained only stickleback DNA and was used directly for amplification with modified universal primers. In addition to these samples, the barcode sequences for four samples (Nos. 29, 35, 125, 173) that did not amplify with species-specific primers for cod, herring, and stickleback and presumably contained lumpfish DNA, were also amplified with modified universal primers.

The validation test resulted in successful sequencing of the barcode DNA sequences for cod and stickleback in samples Nos. 9, 66, 70, 158, and 160. The length of the barcode regions used for comparison was 655 bp.

The barcode sequences for cod (Table 4a) were completely identical in samples No. 66 (accession number in GenBank NCBI – PP087981) and No. 70 (PP087982). The sequence in sample No. 158 (PP087983) differed from these by one base pair (samples Nos. 66, 70 had adenine at 628 bp, while sample No. 158 had guanine, $I = 0.998$). Comparison of these sequences with COI sequences from other cod populations in GenBank NCBI (X99772, HG514359, AM489716, KC015374) also revealed no significant differences ($I = 0.995–1.000$). Sequences HG514359 and AM489716 were identical to those in our samples and only 1–3 nucleotide pairs were different in the other cases ($I = 0.998–0.995$). A detailed analysis of inter- and intraspecific genetic differences is beyond the scope of the present study.

The barcode sequences for stickleback (Table 4b) were identical in samples No. 66 (PP087976) and No. 160 (PP087978). Overall, the greatest differences between all sequenced DNA samples did not exceed 3 base pairs (samples Nos. 9 (PP087975) – 158 (PP087977), $I = 0.995$). Interpopulation differences in threespine stickleback *G. aculeatus* varied significantly ranging from the level of intrapopulation differences of 0–2 bp (sample No. 158 – KX145452, $I = 1.000–0.997$) to 17–20 base pairs (our data – AP002944, $I = 0.974–0.969$). Differences between two species (*G. aculeatus* and *G. wheatlandi*) were as large as 68–70 bp ($I = 0.896–0.893$).

The barcode sequence analysis for cod and stickleback is therefore completely consistent with the previous results obtained using species-specific primers. Among the samples with unclear species identity

Table 4. Identity indices for COI barcode sequences of cod (a), stickleback (b), and (presumably) lumpfish (c).

a

Sample code	X99772 *G. morhua*	HG514359 *G. morhua*	AM489716 *G. morhua*	KC015374 *G. morhua*	66	70	158
X99772	1.000						
HG514359	0.998	1.000					
	(1)						
AM489716	0.998	1.000	1.000				
	(1)	(0)					
KC015374	0.995	0.997	0.997	1.000			
	(3)	(2)	(2)				
66	0.998	1.000	1.000	0.997	1.000		
	(1)	(0)	(0)	(2)			
70	0.998	1.000	1.000	0.997	1.000	1.000	
	(1)	(0)	(0)	(2)	(0)		
158	0.997	0.998	0.998	0.995	0.998	0.998	1.000
	(2)	(1)	(1)	(3)	(1)	(1)	

b

Sample code	NC_011570 *G. wheatlandi*	AP002944 *G. aculeatus*	KX145452 *G. aculeatus*	9	66	158	160
NC_011570	1.000						
AP002944	0.893	1.000					
	(70)						
KX145452	0.893	0.971	1.000				
	(70)	(19)					
9	0.895	0.969	0.998	1.000			
	(69)	20	(1)				
66	0.893	0.971	1.000	0.998	1.000		
	(70)	(19)	(0)	(1)			
158	0.896	0.974	0.997	0.995	0.997	1.000	
	(68)	(17)	(2)	(3)	(2)		
160	0.893	0.971	1.000	0.998	1.000	0.997	1.000
	(70)	(19)	(0)	(1)	(0)	(2)	

c

Sample code	29	125	KX145452 *G. aculeatus*	AM498313 *C. lumpus*
29	1.000			
125	0.996	1.000		
	(2)			
KX145452	0.996	1.000	1.000	
	(2)	(0)		
AM498313	0.801	0.801	0.801	1.000
	(130)	(130)	(130)	

Note. Sample numbers used in this study and sequence accession numbers in GenBank NCBI are shown. Identity indices ($I = 1 - p$-distance) are provided, with the number of base-pair differences between sequences under comparison indicated in parentheses.

(samples Nos. 29, 35, 125, 173), which were amplified with universal barcode primers, only the sequences in samples No. 29 (PP087979) and No. 125 (PP087980) were readable (Table 4c), while those in samples No. 35 and No. 173 were unreadable. Further analysis revealed that the sequences in samples No. 29 and No. 125 differed by 2 base pairs (I = 0.997) and were nearly identical to the COI sequence of *G. aculeatus* (KX145452, I = 0.997–1.000). These sequences differed from the COI sequence of *C. lumpus* in GenBank NCBI (AM498313) on the order of 130 base pairs (I = 0.802). It should be noted that the level of differences between the three fish species (*G. aculeatus*, *G. morhua,* and *C. lumpus*) was as high as 127–143 base pairs and therefore the "readable" barcode sequences in samples No. 29 and No. 125 corresponded to DNA of threespine stickleback. The unreadable sequences (samples No. 35 and No. 173) presumably contained a mixture of DNA from different species, including lumpfish.

Co-occurrence frequencies of DNA extracted from eggs of different fish species

The frequencies of occurrence of stickleback and herring eggs in the cod stomachs varied in different years of the study. In 2017, herring eggs were clearly more numerous in the cod stomachs than stickleback ones (66.7%$_{+23.4\%/-31.8\%}$, and 16.7%$_{+31.7\%/-14.6\%}$, respectively; out of 12 samples), while in 2018, stickleback eggs were prevalent (64.7%$_{+21.1\%/-26.4\%}$, and 35.3%$_{+26.4\%/-21.1\%}$, respectively; out of 17 samples). In general, the frequencies of occurrence of stickleback and herring eggs in the samples from different years of observation (2017–2018) differed from each other by a factor of 2 to 4. Inter-annual differences of occurrence of herring eggs in samples were insignificant (α = 0.168), while the frequency of occurrence of stickleback eggs was higher in 2018 (α = 0.011).

Co-occurrence of stickleback and herring eggs was only found in 2 (samples Nos. 9, 83) out of 29 samples (6.9%, respectively). The analysis of cases of simultaneous occurrence of herring and stickleback eggs in 2017 and 2018 did not reveal significant intra-annual differences between the observed and theoretically expected number of egg co-occurrences for these two species ($\alpha >> 0.05$).

It should be noted that approximately one-third (27.6%$_{+19.7\%/-14.9\%}$) of the examined DNA samples extracted from eggs were contaminated with the host's DNA.

DISCUSSION

In the White Sea, cod inhabits primarily the coastal waters of Kandalaksha Bay, where it plays a significant role in the trophic interactions of hydrobionts in the upper sublittoral zone. Cod has a mixed feeding regime closely linked to the state of its food supply. While fish, particularly threespine stickleback, polychaetes, and crustaceans constitute the primary food groups, the cod summer diet in this bay also includes the eggs of certain fish species. Among these, the most common in the cod diet were the stickleback eggs (Sonina 1957; Bakhvalova et al. 2016), the lumpfish eggs were encountered less frequently (Parukhina 2005; Ershov 2010), and those of herring were only reported once (Yevropeytzeva 1937).

Published data on the diet of cod from the Chupa Inlet area have shown that its stomach contains the eggs of two fish species: lumpfish (Ershov 2010) and stickleback (Bakhvalova et al. 2016). It should be noted that lumpfish eggs (6.5% of the total mass of the stomach content) were shown to be present in the cod diet in the early 2000s (in 2007), i.e. during the period when the stickleback population in the White Sea was still recovering after a prolonged decline that lasted over 40 years. The observations by Bakhvalova et al. (2016) cover a period when the stickleback population in the White Sea had already achieved high levels of abundance (Ivanova et al. 2016). According to Bakhvalova et al. (2016), only stickleback eggs were present in the content of stomachs of cod caught in Chupa Inlet in June, and accounted for up to 50% of the mass of all food items.

Our molecular-genetic analysis of egg samples significantly supplements the existing literature data on the qualitative composition of the cod diet in feeding coastal grounds of the White Sea. First, it has been proven that the cod diet also includes herring eggs. It should be noted that the locations where cod were captured for this study were the same as in previous studies (Ershov 2010; Bakhvalova et al. 2016). Second, the frequency of occurrence of herring eggs in the cod stomachs was rather high, ranging in different years from 35% to 67% of the examined egg samples. This curious fact does not agree with the conclusions of Bakhvalova et al. (2016) that cod predominantly consume large quantities of stickleback eggs. As noted above, reliable species identification by visual examination of eggs from cod stomachs

is not always possible. The application of molecular methods allowed us to establish the species composition and frequency of occurrence of herring and stickleback eggs in the cod's egg diet for this region of the White Sea. The observed interannual variations in the occurrence of herring and stickleback eggs in our material are likely related to the varying availability and abundance of these food components in cod's feeding grounds. Special studies are needed to provide a quantitative assessment of the proportion of each species in the cod summer diet. Third, herring and stickleback eggs have been observed to co-occur in cod stomachs (6.9% of the examined samples), which confirms our field observations that the spawning grounds of these species are located in close proximity to each other in cod fishing areas. It is notable that the present study found a correspondence between the frequencies of actual and expected simultaneous occurrence of eggs of both species. In our opinion, these results can be explained by the fact that the spawning of herring and stickleback overlap significantly in time and that cod show no dietary preferences when consuming the eggs of these fish. Fourth, two out of the 29 examined egg samples contained DNA that did not amplify with species-specific primers for stickleback, herring, and cod, and that could not be identified to species using the barcode regions of the COI gene. Taking into account previously published data on the occurrence of lumpfish eggs in the diet of cod from Chupa Inlet (Ershov 2010), we assume that these samples might contain lumpfish eggs mixed with eggs of stickleback.

Summarizing our findings and literature data, it can be concluded that in the shallow waters of Kandalaksha Bay of the White Sea, fish eggs (stickleback, herring, and lumpfish) provide a readily available and high-energy food resource for cod in June and July, because the abundance of spawning species is relatively high. Stickleback females deposit eggs in nests, with the number of developing eggs in each nest reaching several hundred (Candolin 2004). Herring lay their eggs in several rows as clumps of various sizes on *Fucus, Zostera,* and stones (Soin 1963). The spawning of the lumpfish occurs near the shore among stones and algae (*Fucus*, *Laminaria*) and the deposited clutch of agglutinated eggs has a diameter of up to 10–12 cm (Soin and Mikulin 1974). The dense arrangement of eggs on the substrate, the relative ease of detecting egg clutches on the sea bottom and among algae in the narrow space of the shallow near-shore zone and the presence of extensive spawning grounds for the aforementioned fish species in Kandalaksha Bay (Prozorov 1948; Altukhov et al. 1958; Mukhomedyarov 1966, etc.) contribute to the importance of fish eggs in the cod diet during the summer period in certain areas of the bay.

Despite the presence of eggs from various fish species in the cod diet, current published evidence indicates that cod predominantly consumes stickleback eggs rather than those of lumpfish or herring. This preference can be attributed to the higher abundance of stickleback in coastal waters in June and July compared to the other two species. In fact, threespine stickleback is well known to be the primary food component for cod in Kandalaksha Bay during the spring-summer period (Yevropeytzeva 1937; Sonina 1957; Azarov 1963; Kudersky and Rusanova 1963; Izvekova 1964; Ershov 2010). When huge stickleback shoals appear in coastal waters in June, cod actively switch to consuming both adult fish and the eggs that these fish deposit at the spawning grounds (Sonina 1957). Feeding on herring or lumpfish eggs during this period appears to be non-selective. As cod migrates towards the shores in search of large stickleback aggregations, they inadvertently consume the eggs of herring and lumpfish in shallow waters, along with other food organisms. The predominance of adult stickleback in the cod diet normally persists until stickleback leave the coastal waters in the second half of July.

Like other components of the cod diet in Kandalaksha Bay, the occurrence of fish eggs shows local and interannual variation (Sonina 1957; Izvekova 1964; Ershov 2010; Bakhvalova et al. 2016). This variability is primarily associated with the mosaic distribution pattern of spawning grounds for different species (stickleback, herring, and lumpfish) and the varying intensity of the spawning runs of fish shoals in the coastal waters during different years. For instance, cod individuals caught near Sonostrov in July 1986 had a significant number of fish eggs in their stomachs (26.8% by wet mass), but in July of the same year, no fish eggs were found in the diet of cod that fed in the coastal areas of the Velikaya Salma Strait (Parukhina 2005). During the years of stickleback population decline in the White Sea (from the early 1960s to the late 1990s), this species was entirely absent from the cod diet. By contrast, in years of high stickleback abundance, adult fish and eggs were the primary diet of cod in coastal waters during the sum-

mer season (Sonina 1957; Parukhina 2005; Ershov 2010, etc.). Based on our molecular-genetic data, it can be concluded that herring eggs constitute a part of the cod diet in its feeding grounds in the White Sea. The high frequency of occurrence of herring and stickleback eggs suggests that they play a major role in the diet of cod in the area of Kandalaksha Bay of the White Sea examined in this study. In general, eggs of some fishes (stickleback, herring, lumpfish) are a valuable and high-energy food source for cod in June-July at the coastal waters of the White Sea and can show a comparatively high proportion of the total food intake of cod. Additional seasonal studies on the dynamics of egg occurrence in the stomachs of cod are necessary to provide a more comprehensive assessment of the role played by eggs of different fish species in the summer diet of this predator.

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