Journal of Marine Science and Technology

Volume 31 | Issue 4 Article 15

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Recommended Citation

Sasaki, Kei; Yatsuya, Miwa; Shimizu, Daisuke; Ojima, Daisuke; and Komatsu, Shinji (2023) "Occurrence of Predation of Juvenile Chum Salmon (*Oncorhynchus keta*) by Chub Mackerel (*Scomber japonicus*) and Spotted Mackerel (*Scomber australasicus*) in Miyako Bay, Iwate Prefecture, Japan," *Journal of Marine Science and Technology*: Vol. 31: Iss. 4, Article 15.
DOI: 10.51400/2709-6998.2722
Available at: https://jmstt.ntou.edu.tw/journal/vol31/iss4/15

RESEARCH ARTICLE

Occurrence of Predation of Juvenile Chum Salmon (*Oncorhynchus keta*) by Chub Mackerel (*Scomber japonicus*) and Spotted Mackerel (*Scomber australasicus*) in Miyako Bay, Iwate Prefecture, Japan

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Abstract

In recent years, chub mackerel (*Scomber japonicus*) and spotted mackerel (*Scomber australasicus*) have started migrating to the coastal areas off Iwate Prefecture earlier than they did previously, resulting in a geographic overlap between hatchery-bred juvenile chum salmon (*Oncorhynchus keta*) released at sea and the occurrence of predatory mackerel. To clarify whether the mackerels prey on the juvenile chum salmon, we captured adult mackerels by angling during the period when these species overlap in Miyako Bay, Iwate Prefecture. After capture, the stomach contents of the mackerel were examined using a combination of visual examinations and DNA metabarcoding analysis. As a result, chum salmon were identified in the stomachs of 4 of 97 chub mackerel (May 19), 5 of 30 chub mackerel, and 1 of 7 spotted mackerel (May 26), providing the first evidence of chum salmon predation by mackerels. When the fork length of chum salmon prey was inferred based on otolith measurements, the results showed that the prey items ranged in size from 54.3 mm to 86.9 mm. These findings indicate that the body-size range of the chum salmon that were targeted by mackerels spans that of hatchery-released chum salmon.

Keywords: Diet analysis, DNA metabarcoding, Ocean warming, Otolith

1. Introduction

The chum salmon (*Oncorhynchus keta*) is one of the most economically important fisheries resources in northern Japan, and extensive hatchery production and release of juveniles is conducted to increase stocks. From 2011 to 2020, approximately 1.7 billion hatchery-bred chum salmon were released annually into natural waters in Japan [1]. Hokkaido and the Sanriku region are the main areas for returning adult chum salmon, and Iwate Prefecture has the highest number of returning salmon in the Sanriku region. From 2011 to 2020, approximately 0.33 billion juvenile salmon were released annually from hatcheries into rivers in Iwate Prefecture [2]. However, in 2019 and 2020, the number of adult chum salmon that returned to Iwate Prefecture declined to less than 10 % of the level 10 years earlier. Generally, salmonids are considered to experience high mortality in the early stages of their life history [3,4], and predation is considered to be one of the main causes of high

Received 15 June 2023; revised 9 November 2023; accepted 13 November 2023.
Available online 15 December 2023

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https://doi.org/10.51400/2709-6998.2722

mortality immediately after sea-entry [5,6]. In Japan, known predators of juvenile chum salmon include arabesque greenling (*Pleurogrammus azonus*), Japanese flounder (*Paralichthys olivaceus*), Japanese dace (*Pseudaspius hakonensis*), far eastern dace (*Pseudaspius Brandtii*), spiny dogfish (*Squalus acanthias*), Japanese seaperch (*Lateolabrax japonicus*), pink salmon (*Oncorhynchus gorbuscha*), masu salmon (*Oncorhnchus masou*) and white-spotted charr (*Salvelinus leucomaenis*) [7], as well as pointhead flounder (*Cleisthenes pinetorum*), kurosoi rockfish (*Sebastes schlegelii*) and saffron cod (*Eleginus gracilis*) [8].

Since 2015 in Iwate Prefecture, the catch of mackerel using set nets has increased significantly in April and May, whereas the catch was previously low during this period in earlier years. In the Miyako area of Iwate Prefecture, where the survey of this study was conducted, catches of mackerel using set-nets have also been increasing in April and May [9]. As a result, the overlap between the period of coastal migration of mackerel and the period of coastal residence of juvenile chum salmon, which are released mainly in April and May, increases the likelihood of encounters between the salmon and mackerel in the wild. Two species of mackerel are distributed along the coast of Iwate Prefecture, chub mackerel (*Scomber japonicus*) and spotted mackerel (*Scomber australasicus*), both of which belong to Pacific stocks (referred to collectively hereafter as "mackerels"). Although mackerels prey upon a variety of organisms, they are primarily piscivorous. In the northeastern Pacific Ocean, chub mackerel feed extensively on Japanese anchovy (*Engraulis japonicus*), as well as on small crustaceans, such as copepods and krill [10]. Japanese anchovy has also been identified in the stomach contents of spotted mackerel [11]. Mackerels are considered to be potential predators of juvenile salmon during their northward migration. Indeed, the migration and feeding patterns of predatory fishes can both have a negative impact on prey fish stocks [12−14]. Since mackerels are relatively abundant in the coastal waters off the Sanriku region, intense predation of juvenile chum salmon by mackerels could be one of the factors that account for the decline in chum salmon stock. However, there is no scientific evidence to show that mackerels prey on juvenile salmon in the region.

To clarify whether juvenile chum salmon are preyed upon by mackerels in the field, this study examined the stomach contents of mackerels caught in Miyako Bay, Iwate Prefecture, Japan. To determine whether the fish identified by observations of stomach contents were chum salmon, the fish species in gut contents were identified to species using DNA analysis. We also evaluated the feeding habits of mackerel using DNA metabarcoding to assess the relative importance of juvenile chum salmon among prey species. In addition, otoliths from chum salmon retrieved from mackerel gut contents were used to estimate the body length of prey.

2. Materials and methods

2.1. Samples

Mackerel specimens in Miyako Bay, Iwate Prefecture, were sampled by angling on five days (April 9, April 20, May 19, May 26, and June 1) in 2021 during the period when juvenile chum salmon inhabit coastal areas. Sampling was conducted at the central part of Miyako Bay and at the mouth (Fig. 1), as these areas were assumed to be where juvenile chum salmon would first encounter mackerels after being released. Since chub mackerel do not feed at night [11], and the stomach contents of Atlantic mackerel (*Scomber scombrus*) have been reported to decrease from evening to sunrise [15], all sampling was conducted after sunrise, from 7:00 a.m. to approximately noon. Mackerels were caught by pole and lure fishing on a fishing boat fitted with a fish finder (FCV-1100L, Furuno, Nishinomiya, Japan). Collected fish were placed immediately in a cooler box containing seawater and ice.

The specimens were then transferred to the Miyako Field Station of the Japan Fisheries Research and Education Agency (FRA) (Miyako, Iwate, Japan) and processed on the same day. Specifically, the mackerel species were identified based on the ratio of the basal length of spines 1−9 of the first dorsal fin to the fork length (FL) [16], with all measurements performed to the nearest 1 mm. Body weight (BW) was measured to the nearest 1 g. Stomachs were dissected with tweezers and scissors and the contents were placed into a sterile Petri dish and weighed to the nearest 0.01 g. The stomach contents were then visually identified to the lowest taxonomic level possible (i.e., genus or species). To prevent cross contamination of samples, tweezers and scissors were cleaned thoroughly using RNase AWAY (Thermo Fisher Scientific, Waltham, MA, USA) between each dissection.

To identify partially digested fish prey in the stomach contents to species (Fig. 2), approximately 100 mg of muscle tissue from each of 30 fish prey items, which were either visually identified or suspected of being chum salmon, was collected using dissection instruments cleaned with RNase AWAY. Thereafter, all of the stomach contents, including any remaining fish muscle tissue samples, were pooled

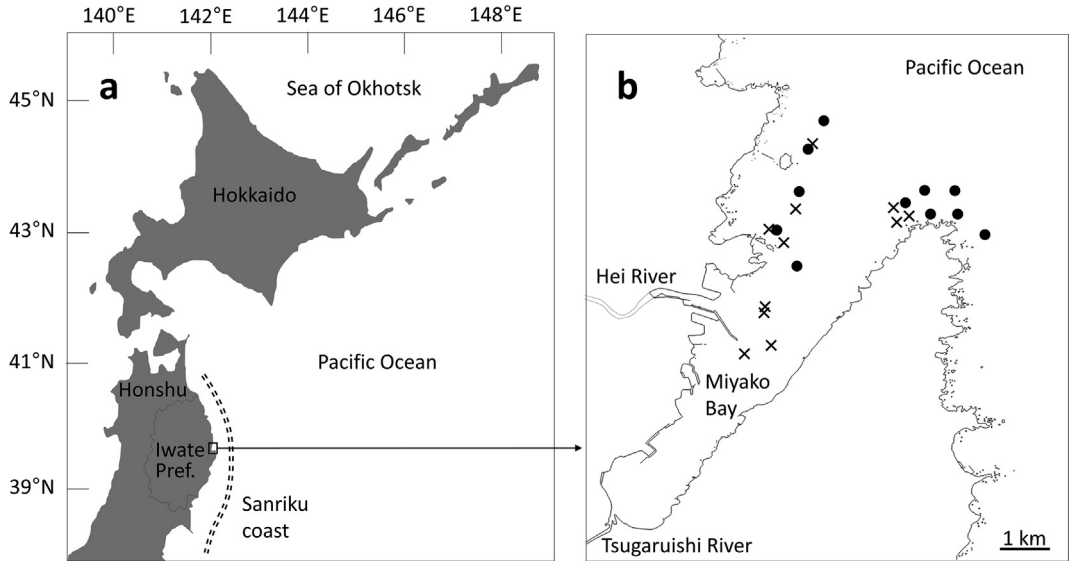

Fig. 1. Maps showing the location of the (a) Sanriku region in northern Japan and (b) the sampling area in Miyako Bay (inset). Locations of pooled survey points where mackerels were either captured (solid circles) or not captured (crosses) during the survey period.

on each angling day and homogenized on ice using a homogenizer (T25 Digital, IKA, Staufen, Germany). The prey muscle tissues and the homogenized stomach contents were stored at −80 °C until DNA extraction. For each fishing day, the weight percentage of chum salmon in the stomach contents of mackerels was calculated by dividing the weight of the chum salmon by the weight of the total stomach contents of the mackerels in the pooled sample.

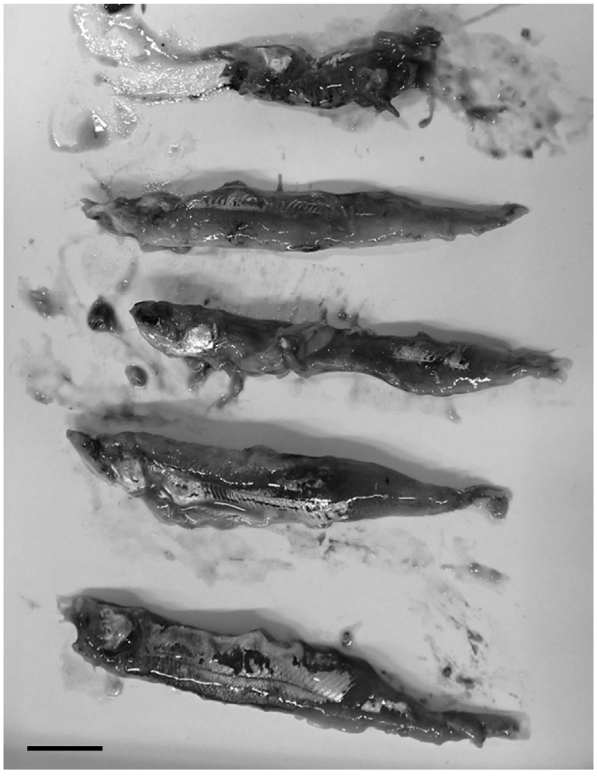

Fig. 2. Representative example of mackerel stomach contents comprising juvenile chum salmon identified based on visual observation. Scale bar: 10 mm.

2.2. DNA extraction

DNA extraction, DNA library preparation, Illumina Miseq sequencing (Illumina, San Diego, CA, USA), and sequence processing were performed by Bioengineering Lab. Co., Ltd. (Kanagawa, Japan) as follows. Crude DNA was extracted from the muscle tissue using Lysis Buffer for PCR (TaKaRa Bio, Kusatsu, Japan). The homogenized stomach contents were lyophilized and pulverized using a freeze dryer (VD-250R, TAITEC, Koshigaya, Japan) and a bead-type homogenizer (Multi-bead Shocker, Yasui Kikai, Osaka, Japan). DNA was extracted from the stomach contents using Lysis Solution F (Nippon Gene, Tokyo, Japan) and an MPure Bacterial DNA Extraction Kit (MP Bio Medicals, Irvine, CA, USA). The DNA concentrations except for crude DNA were measured using a QuantiFluor dsDNA System (Promega, Madison, WI, USA) and a microplate reader (Synergy H1, Agilent Technologies, Santa Clara, CA, USA).

2.3. DNA library preparation and MiSeq sequencing

The cytochrome *c* oxidase subunit 1 (CO1) region of mitochondrial DNA was used for DNA metabarcoding analysis. DNA libraries for the MiSeq

platform were prepared using a two-step tailed PCR method. The first PCR for the muscle samples from prey fish was performed in a 20 μL volume containing 0.4 μL of Gflex DNA polymerase (TaKaRa Bio), 10 μL of 2 × Gflex PCR buffer, 0.5 μL of each primer (10 μM each), 7.6 μL of sterile distilled water, and 1.0 μL of template DNA. The fragments of the CO1 genes were amplified using a universal metazoan primer set [17,18] that incorporated the forward and reverse adapter sequences (5′-ACACTCTTTCCCTACACG ACGCTCTTCCGATCT-GGWACWGGWTGAACW GTWTAYCCYCC-3′ and 5′-GTGACTGGAGTTCA GACGTGTGCTCTTCCGATCT-TAHACTTCNGGG TGKCCRAARAATCA-3′) used in the second PCR. The thermal cycle conditions consisted of 94 °C for 1 min, followed by 35 cycles of 98 °C for 10 s, 55 °C for 15 s, and 68 °C for 1 min, and finally 68 °C for 5 min. The first PCR reactions of the stomach content samples used the same primer set that was used for the muscle samples. The PCR reactions were performed in a 10 μL volume containing 0.08 μL of Ex Taq HS (TaKaRa Bio), 1.0 μL of 10 × Ex Taq Buffer, 0.8 μL of dNTPs (2.5 mM each), 0.5 μL of each primer (10 μM each), 4.0 μL of blocking primer (10 μM), 1.12 μL of sterile distilled water and 2.0 μL of template DNA (2 ng/μL). The blocking-primer (5′-AAACCCCTCTGTTCGTCTGAGCAGTCC/3SpC3/-3′) was used to reduce the number of reads derived from the mackerels' own DNA. The thermal cycle conditions consisted of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 61 °C for 15 s, 52 °C for 30 s, and 72 °C for 30 s, and finally 72 °C for 5 min. The first PCR products of both samples were purified by AMPure XP bead-based DNA purification (Beckman Coulter, Brea, CA, USA) and the DNA concentrations were measured.

The second PCR reactions for both experiments were performed in a 10 μL volume containing 0.1 μL of ExTaq HS, 1.0 μL of 10 × Ex Taq Buffer, 0.8 μL of dNTPs (2.5 mM each), 0.5 μL of each primer (10 μM each), 5.1 μL of sterile distilled water and 2.0 μL of template DNA (muscle samples; <1.3 ng/μL, stomach content samples; 4.6 or 5.0 ng/μL). The sequences of the forward and reverse primers were 5′-AATGATACGGCGACCACCGAGATCTA-CAC-Index2-ACACTCTTTCCCTACACGACGC-3′ and 5′-CAAGCAGAAGACGGCATACGAGAT-Index1-GTGACTGGAGTTCAGACGTGTG-3'. For accurate recognition of the samples, the index pair was specific to each sample. The thermal cycle conditions consisted of 94 °C for 2 min, followed by 10 or 12 cycles of 94 °C for 30 s, 60 °C for 15 s, and 72 °C for 30 s, and finally 72 °C for 5 min. The second PCR products were purified by AMPure XP bead-based DNA purification. The concentration of the DNA in each DNA library was 0.6−18.6 ng/μL for the muscle samples and 21.1−25.0 ng/μL for the stomach content samples. The quality of the libraries was confirmed using a fragment analyzer and a dsDNA 915 Reagent Kit (Agilent). The library was pair-end sequenced (2 × 300 bp) on a MiSeq sequencer with a MiSeq v3 Reagent Kit (Illumina).

2.4. Sequence processing

Complete tag-matching sequences were extracted from the forward and reverse raw-data sequence files using the fastaq barcode splitter implemented in the FASTX-Toolkit (ver. 0.0.14) [19] with the primer sequences removed. Extracted sequences were then trimmed and sequences with quality scores of <20 and lengths of <40 bp were discarded using Sickle Tools (ver. 1.33) [20]. Trimmed forward and reverse sequences with a merged length of 310 bases, reading length of 225 bases and minimum overlap size of 10 bases were then merged using FLASH (ver. 1.2.11) [21] and the merged sequences from the muscle samples were then subjected to homology searches using BLASTN (ver. 2.9.0). For the homogenized stomach contents, the merged sequences were grouped into operational taxonomic units (OTUs) using QIIME 2 [22] with default settings. Phylogenetic relationships were inferred based on BLAST searches of representative OTU sequences against the NCBI nt database. Taxa with read counts exceeding 100 OTUs were regarded as a species. The obtained sequence data were deposited in the DDBJ under BioProject accession numbers DRR353432−DRR353435.

2.5. Estimation of FL of chum salmon in stomach contents

In the event that direct measurement of the FL of the prey items that were identified by DNA analysis as being chum salmon was not possible due to the specimen being partially digested (Fig. 2), the radius of otoliths extracted from the remaining head was measured to the nearest 1 μm, and the FL was inferred based on the relationship between otolith radius and the FL of juvenile chum salmon, as reported by Saito et al. [23]. The following formula (1) was used to estimate *FL*.

$$FL = a \times exp\,(b \times R) \tag{1}$$

Here, R is the otolith radius, and a and b are constants. The values for the constants were taken from Saito et al. [23], and were 11.2 and 4.97 × 10^{-3}, respectively. The FL of the chum salmon prey and

mackerels was then compared to examine the predator-prey size relationship.

2.6. Confirmation of chum salmon otolith thermal markings in mackerel stomach contents

To determine if the chum salmon prey originated from hatcheries, the presence of otolith thermal marks was examined. The total number of chum salmon released from hatcheries in Iwate Prefecture in 2021 was 232 million [2], of which 28 million (12 %) were subjected to otolith thermal marking [24].

3. Results

A total of 166 mackerel were caught on all sampling days except on April 20. All of the specimens were caught near the mouth of Miyako Bay (Fig. 1) at water depths ranging from 29.0 to 74.0 m. Of all the captured mackerel, the number of individuals (percentage) of chub mackerel was 153 (92.2 %) and that of spotted mackerel was 13 (7.8 %). The mean FL ± SD (range) of the chub and spotted mackerels were 324 ± 26 (185–432) mm and 323 ± 30 (256–360) mm, respectively (Table 1).

DNA analysis of the muscle tissue of prey items revealed that the total raw read count of each individual ranged from 14341 to 25062, and a search for the highest number of merged sequences showed that 29 out of 30 individuals were identified as chum salmon with more than 99 % similarity (DDBJ/EMBL/GenBank databases under accession numbers LC094478.1, LC09471.1, LC094479.1). Juvenile chum salmon were found in the stomachs of 4 out of 97 chub mackerel collected on May 19, and no juvenile chum salmon were found in the stomachs of three spotted mackerel on this date. On May 26, juvenile chum salmon were found in the stomachs of 5 out of 30 chub mackerel and in 1 out of 7 spotted mackerel (Tables 1 and 2). No juvenile chum salmon were found in the stomachs of chub and spotted mackerels collected on April 9 and June 1. The weight percentage of chum salmon in the pooled stomach contents of mackerels collected on May 19 and May 26 was 2.5 % and 29.0 %, respectively. Other fish species caught in the bay included fat greenling (*Hexagrammos otakii*), roundnose flounder (*Eopsetta grigorjewi*), white-edged rockfish (*Sebastes taczanowskii*), fox jacopever (*Sebastes vulpes*), long shanny (*Stichaeus grigorjewi*), but visual observations of the stomach contents of these fishes did not reveal any chum salmon.

DNA analysis of the stomach contents of the mackerels pooled by catch date showed that total OTU read counts with more than 97 % similarity ranged from 40230 to 57113, of which 1286 to 9421 were host mackerel OTU reads, which in turn accounted for 2.4–23.4 % of the total (Table 3). The results of the mackerel diet DNA analysis showed that mackerels preyed on fishes such as Japanese sardine (*Sardinops melanostictus*), Japanese anchovy, chum salmon, chub mackerel, walleye pollack (*Gadus chalcogrammus*), and white-spotted conger (*Conger myriaster*). Japanese anchovy and chub mackerel were detected on all sampling days. As for invertebrates, amphipods such as *Jassa* spp. and *Caprella* spp., euphausiids such as *Euphausia pacifica*, and Cnidarians were also detected (Table 3). The taxa identified by visual observation included Japanese sardine, Japanese anchovy, chum salmon, white-spotted conger (leptocephalus), squids, decapods (Megalopa), and euphausiids and amphipods (*Jassa* sp. and *Caprella* sp.), all of which were also identified by DNA analysis (Table 3). Although mackerels were detected in all of the DNA samples on all of the sampling days, no mackerels were observed by visual observations of the gut contents.

The mean FL of juvenile chum salmon estimated from otoliths ± SD (range) was 66.4 ± 10.0 (54.3–86.9) mm (Table 2). There was no statistically significant correlation between the FL of the predators and the chum salmon prey (Pearson's correlation, $r = 0.063$, $p = 0.809$). Of the 29 chum salmon samples in the stomach contents of mackerels, otoliths from 13 specimens could not be analyzed

Table 1. Date of sampling, fork length, and body weight of the chub and spotted mackerels.

Sampling date	Mackerel species	n	Mean ± standard deviation (range) of fork length (mm)	Mean ± standard deviation (range) of body weight (g)
April 9	chub	17	285 ± 20 (255–320)	236 ± 60 (147–374)
	spotted	0	–	–
April 20	chub	0	–	–
	spotted	0	–	–
May 19	chub	97	326 ± 25 (185–363)	343 ± 79 (54–534)
	spotted	3	331 ± 20 (313–359)	403 ± 80 (330–514)
May 26	chub	30	318 ± 23 (264–352)	303 ± 67 (184–421)
	spotted	7	329 ± 20 (292–350)	392 ± 85 (270–504)
June 1	chub	9	332 ± 41 (270–432)	394 ± 205 (215–948)
	spotted	3	300 ± 44 (256–360)	365 ± 177 (203–611)

Table 2. Body size of chub and spotted mackerels that preyed upon juvenile chum salmon, and body size and presence of otolith thermal marking of chum salmon preyed upon by the mackerels.

Sampling date	Mackerels				Ratio of chum salmon weight to mackerel stomach content weight (%)	Chum salmon			
	No.	Species	Fork length (mm)	Body weight (g)		No.	Fork length (mm)	Sample weight (g)	Otolith-marked
May 19	1	Chub	312	296	13.3	1	57.2[a]	2.3	No
	2	Chub	335	342	34.3	2	74.1[a]	4.6	No
	3	Chub	345	369	50.0	3	ND	2.4	
	4	Chub	334	387	31.2	4	59.5[a]	2.4	No
May 26	5	Chub	332	375	52.4	5	58.5[a]	2.3	No
						6	54.3[a]	2.8	No
						7	66.7[a]	2.4	No
						8	72.5[a]	2.7	No
						9	57.8[a]	1.6	Yes
	6	Chub	302	275	25.4	10	ND	1.5	
	7	Chub	342	393	92.1	11	ND	1.4	
						12	ND	1.9	
						13	ND	1.4	
						14	ND	1.3	
						15	ND	2.1	
						16	ND	1.5	
						17	ND	2.0	
	8	Chub	348	392	94.8	18	54.3[a]	0.6	No
						19	70.1[a]	2.4	No
						20	ND	1.8	
						21	ND	0.8	
						22	ND	1.3	
						23	ND	2.3	
	9	Spotted	350	499	82.9	24	75.8[a]	3.0	Yes
						25	71.3[a]	2.1	Yes
						26	66.3[a]	1.9	No
						27	57.5[a]	1.0	No
						28	86.9[a]	2.7	No
	10	Chub	310	269	81.8	29	82.8[a]	3.6	No

[a] fork length estimated from otolith radius; ND: no data.

Table 3. Prey species in stomach contents of mackerels on each collection day estimated by DNA metabarcoding.

Species	Common name	April 9		May 19		May 26		June 1	
Fishes									
Oncorhynchus keta	Chum salmon	0		4679	v	11734	v	0	
Engraulis japonicus	Japanese anchovy	14378	v	18709	v	4023	v	6660	v
Sardinops melanostictus	Japanese sardine	474		12841	v	<100		10803	v
Scomber japonicus	Chub mackerel	9421		1351		1286		2644	
Gadus chalcogrammus	Walleye pollock	1060		<100		<100		0	
Leuroglossus schmidti	Northern smooth tongue	831		0		0		0	
Conger myriaster	White-spotted conger	0		0		686	v	272	v
Pterogobius zacalles	Beauty goby	0		0		0		1428	
Physiculus japonicus	Japanese codling	0		0		200		0	
Gadus macrocephalus	Pacific cod	230		0		0		0	
Diaphus theta	California headlight fish	0		444		0		0	
Microstomus achne	Slime flounder	130		0		0		0	
Molluscs									
Squid									
Todarodes pacificus	Japanese flying squid	0		5480	v	<100		111	
Crustaceans									
Decapoda									
Telmessus cheiragonus	Helmet crab	0		<100		136	v	<100	
Euphausiidae									
Euphausia pacifica	Pacific krill	11890	v	308	v	<100		0	
Copepoda									
Acartia sp.	-	0		0		0		197	v
Podonidae									
Evadne nordmanni	-	0		<100		218		<100	
Amphipoda									
Jassa spp.	-	0		2637	v	13662	v	8250	v
Caprella spp.	-	0		738	v	4715	v	10168	v
Polychaetes									
Nicolea sp.	-	0		0		0		1609	
Echinodermata									
Sclerodactyla multipes	-	0		0		0		434	
Cnidarians									
Eutonina indicans	Umbrella jellyfish	787		6757		0		0	
Agalma elegans	-	407		<100		0		0	
Sarsia tubulosa	Clapper hydroid	0		101		0		<100	
Aequorea sp.	-	0		1949		0		0	
Algae									
Gloiopeltis sp.	-	0		0		1197		0	
Analipus japonicus	Far needle	0		<100		655		<100	
Stephanocystis geminata	Chain bladder	0		0		138		<100	
Chloroparvula sp.	-	113		0		0		0	
Others		509		1119		1644		1087	
Total		40230		57113		40294		43663	

Values indicate the read counts of operational taxonomic units (OTU), and "v" indicates taxa that were confirmed at the species or genus level by visual observation. Taxa with read counts less than 100 or taxa with no "hits" the in the NCBI nt database were grouped as "Others".

because the heads were completely digested and otoliths were scattered throughout the stomach contents.

The results of otolith thermal marking analysis showed that, three of 16 (18.8 %) individuals were marked (Fig. 3, Table 2).

4. Discussion

2The findings of this study showed that mackerels prey on juvenile chum salmon in Miyako Bay. In coastal areas of Japan, nine fish predators of juvenile chum salmon were reported by Nagasawa [7] and three fish predators by Miyakoshi et al. [8]; however, chub and spotted mackerels were not among these species. These two mackerel species are thus newly described as fish predators of juvenile chum salmon. The detection of juvenile chum salmon in stomach contents is frequently based on visual observations, which are often not sufficiently accurate to identify them to the species level, except for cases where individuals have been ingested only shortly before the stomach contents are examined [8]. In this study, a combination of visual examination and

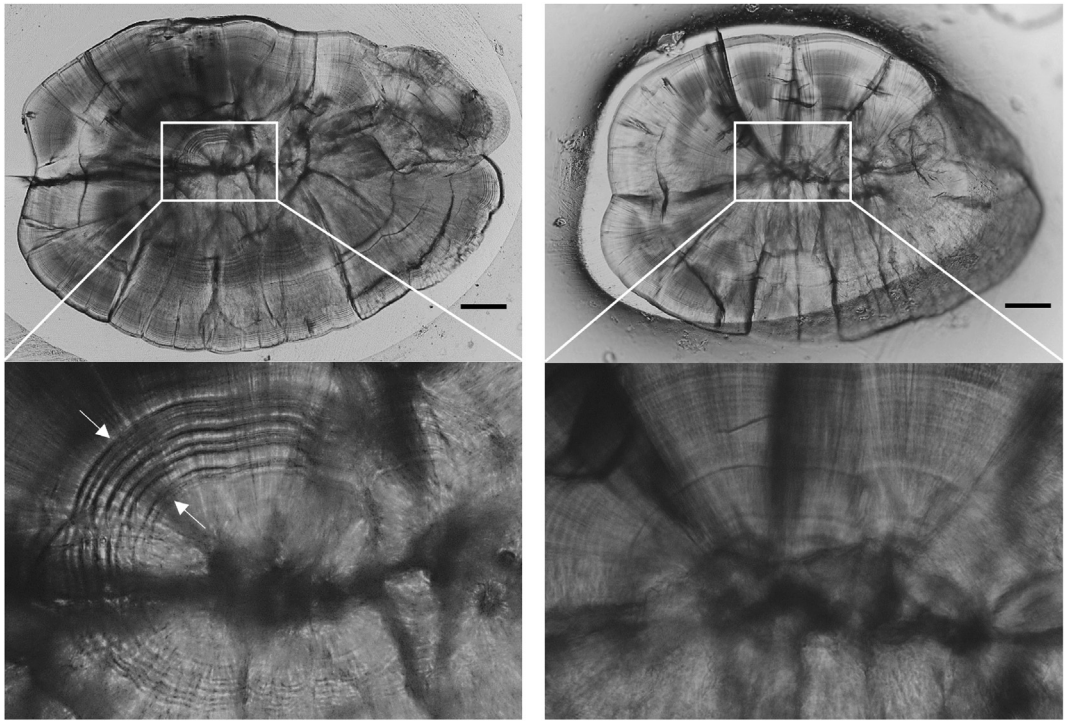

Fig. 3. Examples of otoliths of chum salmon preyed upon by the mackerels with (left) and without (right) otolith thermal markings (between the white arrows). Scale bar: 100 μm.

DNA analysis enabled us to accurately identify prey items.

Mackerels preying on juvenile chum salmon were caught at the mouth of Miyako Bay at depths ranging from 34.0 to 68.3 m. Most of the juvenile chum salmon collected from the mackerel stomachs were undigested, and the epidermis and subepidermal muscle tissues remained intact (Fig. 2), suggesting that the chum salmon had been preyed upon in the waters near the catch site. It is thus possible that juvenile chum salmon from rivers flowing into Miyako Bay were preyed upon as they migrated out of the bay, and/or that juveniles from regions nearby encountered mackerels near the bay mouth during their northward migration along the coast. Juvenile chum salmon generally swim near the sea surface [25]. Chub mackerel migrate diurnally from depths ranging between 0 and 130 m, staying mainly in deep water during the day [26]. Spotted mackerel frequently move vertically from the surface to depths of 50 m depending on the season [27]. Consequently, the predation of juvenile chum salmon is considered to occur near the sea surface.

The FL of juvenile chum salmon collected from mackerel stomachs ranged from 54.3 to 86.9 mm. Approximately 25 million juvenile/young chum salmon were released into Tsugaruishi River, which flows into Miyako Bay, from mid-March to early May in 2021. Most of the released juveniles had a mean fork length between 44.9 and 72.0 mm (Miyako Fishery Cooperative Association, pers. comm., 2021). Thus, many of the released juvenile chum salmon were smaller than the largest juvenile chum salmon prey (FL, 86.9 mm) observed in the stomach contents of mackerels. These findings indicate that the range in body size of chum salmon in the mackerels covers the range of hatchery-released chum salmon, and suggest that the predatory capacity of mackerels is high in terms of prey size.

In this study, three otolith-marked chum salmon were identified among the mackerel prey, indicating that hatchery-released chum salmon are included in the predated fish. However, since not all of the released fish were marked, it is not known whether, and what percentage of, the non-marked individuals originated from hatcheries or the wild. Isotope analysis is often used to discriminate between hatchery-bred and wild salmonids [28–30]. In future research, this technique will enable assessment of the proportion of released fish among the chum salmon prey.

Up to 16.7 % (5 out of 30) and 14.3 % (1 out of 7) of the chub mackerel and spotted mackerel, respectively, had juvenile chum salmon in their stomachs on each sampling day (Tables 1 and 2). Mackerels also preyed on organisms other than chum salmon (Table 3). A previous study reported that mackerels feed on a variety of organisms, including fish and

zooplankton [11], and the findings of the present study corroborated these findings (Table 3). On the other hand, even though no mackerel prey were observed in the visual examinations of stomach contents, DNA metabarcoding detected mackerel on all of the sampling days. It therefore seems highly likely that host-derived mackerel DNA was detected. Although blocking primers that suppress host-derived DNA were used in this study, they do not completely suppress it. Indeed, the risk of false positives is always prevalent when performing diet analysis using DNA metabarcoding. We compensated for this potential limitation of the study by identifying the prey organisms based on simple visual morphological observations before DNA metabarcoding analysis.

Further clarification of the quantitative impact of mackerel predation on the survival of juvenile chum salmon is necessary. An abundance of prey organisms likely affects the predation pressure of mackerels on juvenile chum salmon. In the case of birds that prey on juvenile salmonids in coastal waters, it is known that predation pressure on juvenile salmonids increases when there are relatively few other prey species, but it decreases when prey species are abundant [31,32]. Therefore, in addition to clarifying the spatiotemporal characteristics of the overlap in the distribution of the juvenile chum salmon and mackerels, it is necessary to determine how changes in the biotic and abiotic environment of the mackerels affect feeding pressure on chum salmon.

Funding

This research was funded by the Japan Fisheries Research and Education Agency (FRA).

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

Acknowledgements

We thank S. Imai, T. Horanai, A. Nakashima, and Y. Yokoda of the Miyako Field Station of the FRA for their cooperation with mackerel sampling and measurements. We also thank the captain of the fishing boat “Heishin Maru” for providing some of the mackerel specimens.

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