

FULL PAPER

Wildlife Science

Population- and growth-related differences in helminthic fauna of finless porpoises (Neophocaena asiaeorientalis) in five **Japanese populations**

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A survey of helminths and ectoparasites, including epizoits, was conducted in ABSTRACT. narrow-ridged finless porpoises (Neophocaena asiaeorientalis) from Japanese five populations using dead stranded or incidentally caught animals. In total, 13 helminth species were found (6 nematodes, 4 trematodes, 2 cestodes and 1 acanthocephalan) in 137 porpoises. A new location record of Stenurus nanjingensis and a new host record of Tetrabothrius sp. were obtained. Eight species of helminth were considered common in the Japanese populations of the finless porpoise: Pharurus sunameri, Pharurus asiaeorientalis, Nasitrema spathulatum, Nasitrema sunameri, Halocercus pingi, Halocercus sunameri, Campula oblonga and Synthesium elongatum. No anisakid nematodes were found. N. spathulatum was found only in the western waters of the Seto Inland Sea. Low prevalence of C. oblonga in the Omura Bay was demonstrated. H. pingi was mostly found in very young porpoises before starting to eat prey, indicating prenatal or transmammary infection. However, a congeneric species, H. sunameri, mainly infected weaned porpoises, indicating that these two species possess different transmission pathways. This study provides information on the geographical distribution and prevalence of helminth parasites in finless porpoises off the Japanese coast.

KEY WORDS: finless porpoise, helminthic fauna, population, prevalence

The narrow-ridged finless porpoise (Neophocaena asiaeorientalis) is a small odontocete distributed in eastern Asia, from the Taiwan Strait to the Korean and Japanese coasts [15]. In Japanese waters, five discrete populations have been recognized by morphological and genetic differences (Fig. 1) [40, 41]. These are the Omura Bay (OB), Ariake Sound-Tachibana Bay (AT), Seto Inland Sea (SIS), Ise-Mikawa Bays (IM) and Tokyo Bay-Sendai Bay (TS) populations.

Parasites of the finless porpoise in Japanese waters have been studied by several authors. In early studies, some new helminth species were described based on the examination of a few hosts [24, 37, 38]. Subsequently, a parasitological study was conducted to understand the faunal differences between different host populations [21]. However, these studies only examined hosts from the SIS and TS populations; no parasitological studies have been conducted on the OB, AT and IM populations. In addition, the number of hosts examined was limited, and no information about the prevalence of parasitic infection was obtained, even in the previously studied populations.

In this study, helminths and ectoparasites, including epizoits, of the narrow-ridged finless porpoise were surveyed in five populations in Japanese waters using animals found dead on the beach (stranding) or incidentally entangled in fishery nets (bycatch). The aims of this study were to reveal the parasitic fauna of the OB, AT and IM populations, as well as to further examine the SIS and TS populations; to investigate the differences between them; and to provide the prevalence of parasitic infection in finless porpoises off the Japanese coast.

MATERIALS AND METHODS

The narrow-ridged finless porpoises examined in this study were collected during the period from 2010 to 2015 from the following areas: the Omura Bay, Ariake Sound, Tachibana Bay, Seto Inland Sea, Ise Bay, Mikawa Bay and the Pacific coast of Chiba and Ibaraki Prefectures. The host animals were autopsied at the stranding site or transported to the laboratory and frozen in

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Fig. 1. Locations of the five known Japanese populations of the finless porpoise.

Location (Dopulation)	Sompling pariod	Number of onimals	C	ategory based of	on body leng	th
Location (Population)	Samping period	Number of animals	<90 cm	90–110 cm	>110 cm	Unclear
Omura Bay (OB)	2010-2015	50	17	7	26	-
Ariake Sound, Tachibana Bay (AT)	2010-2015	42	6	10	25	1
Seto Inland Sea (SIS)	2012-2015	24	5	2	17	-
Ise Bay, Mikawa Bay (IM)	2014	19	14	0	5	-
Pacific coast of Chiba and Ibaraki prefecture (TS)	2013-2014	2	1	0	1	-
Total		137	43	19	74	1

-18°C, to be autopsied on another day. In total, 137 porpoises were used in this study; the host information is shown in Table 1.

The parasitological examination followed the methodology described previously [21]. The surface of the host animal was investigated before the autopsy to search for ectoparasites and epizoits. The lungs and liver were cross-sectioned and observed macroscopically, then sliced into 1 to 2 cm thickness and washed in fresh water with slight pressure by hands. The stomach and intestine were opened entirely and washed in fresh water. The cranial sinus was opened and rinsed in running water. All of the washes obtained were left to stand, and then, the precipitation was observed under a stereomicroscope to collect any helminths. In some cases, this method could not be applied to all organs and tissues owing to post-mortem change, including organ loss or degradation. Only cross-sectioning and macroscopic observations were applied to highly degraded organs, and extremely degraded organs were not examined. Totally cranial sinus of 118, lungs of 114, liver of 69, stomach of 49 and intestine of 88 porpoises were examined. When parasites were found in other tissues or organs during the autopsy, they were also collected.

Nematodes were fixed in 70% or 99% ethanol, or 10% neutral buffered formalin, and cleared for observation using Gater's solution. The worms were identified morphologically and counted. The nematodes in the cranial sinus were often very numerous. In such cases, the total number of worms was estimated based on the proportion of the weight of the worms [21]. One hundred entire worms were randomly selected from the collected worms and identified; then, the wet weights of the 100 worms and the total worms were measured. The number of worms of each identified species was estimated from its proportion in the sample, using the following equation: (number of identified worms)×(total worms weight)/(100 worms weight). The identification of the female lungworm was very difficult owing to morphological similarity between species; therefore, only male lungworms were identified.

Trematodes, cestodes and acanthocephalans were flattened between a pair of slide glasses and fixed in 70% ethanol. After fixation, the worms were stained with alum-carmine, Semichon's carmine or Heidenhain's iron hematoxylin, and mounted in Canada balsam. They were then identified morphologically and counted.

The identification was based on species descriptions or identification keys. All specimens are deposited in the Marine Mammal Research Laboratory, Nagasaki University (NU MMRL Parasite Collection Nematoda 2–21, 24, 28–31, 33–44, 46, 47, 50–91, 96–100, 104–114, 118–125, 128, 129, 132, 133, 136–193, 195–207, 213; Digenea 4–13, 18–20, 22–24, 28–40, 42–53, 56–58, 72–83, 88, 89, 91, 92, 95–124, 127–132, 135–149, 151, 152; Cestoda 1, 9, 69, 71; Acanthocephala 3).

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Class	Predominant site of	Other sites	References for identification
Species	infection	other sites	References for identification
Nematoda			
Pharurus sunameri	cranial sinus	trachea, lung	[38]
P. asiaeorientalis	cranial sinus	trachea	[27]
Stenurus nanjingensis	cranial sinus		[34]
Halocercus pingi ^{a)}	lung	trachea, uterus	[26, 36]
H. sunameri ^{a)}	lung		[38]
<i>H. taurica</i> ^{a)}	lung		[4]
Trematoda			
Nasitrema sunameri	cranial sinus		[37]
N. spathulatum	cranial sinus		[24]
Campula oblonga	liver/bile duct	stomach, intestine	[37]
Synthesium elongatum	intestine	stomach, duodenum	[24]
Cestoda			
Diphyllobothrium fuhrmanni	intestine		[18]
Tetrabothrius sp.	intestine		[13]
Acanthocephala			
Corynosoma sp.	intestine		[21]

a) Only male worms were identified.

The prevalence of infection was calculated based only on the organs/tissues that had been washed and microscopically observed. Many helminths with indirect life cycles are transmitted to their definitive host through dietary ingestion, including ingestion of infective larvae. Therefore, whether the host has started to eat prey or not must be considered for the prevalence calculation. Based on logistic models, Shirakihara *et al.* [31] estimated that narrow-ridged finless porpoises start to eat solid food and wean at 98 cm and 101 cm in body length, respectively. In their models, a porpoise is very unlikely (5%) to be consuming solid food when it is 92 cm long, and to be consuming milk when it is 109 cm long. Therefore, in this study, <90 cm long porpoises were regarded as nursing (do not eat prey) and >110 cm long porpoises were considered weaned (only eat prey). Porpoises of 90–110 cm body length were regarded as weaning (in transition between nursing and weaned). The prevalence of infection was calculated separately for these three growth categories.

The differences in prevalence among host populations or between growth categories were tested using Fisher's exact test. Pairwise Fisher's exact test with the Benjamini–Hochberg procedure was used for multiple comparisons between host populations. All statistical analysies were performed using R, version 3.3.1 [29], and the statistical package fmsb [23] was used in multiple comparisons.

RESULTS

The identified species and sites of infection are shown in Table 2. In total, 13 species of helminths, 6 of nematodes, 4 of trematodes, 2 of cestodes and 1 of acanthocephalans were found. No ectoparasites or epizoits were found. Most of the helminths were consistently found in a specific infection site; however, some worms were in other sites. *Pharurus sunameri* and *Pharurus asiaeorientalis* were mainly found in the cranial sinus, with a few worms occurring in the respiratory organs. *Halocercus pingi* was very long; in cases of severe infection, the worms reached from the bronchiole to the trachea. *H. pingi* was also found in the uterus of one young porpoise. *Campula oblonga* and *Synthesium elongatum* were found in the liver/bile ducts and the intestine, respectively. These trematodes were also found in the stomach, but no other helminths occurred in the stomach. The detailed investigation and identification of *Tetrabothrius* sp. were suspended, because only a single specimen was obtained. The general appearance of *Corynosoma* sp. was consistent with the description of unidentified specimens by Kuramochi *et al.* [21].

Table 3 shows each helminth species with the number of infected hosts, and the estimated mean, minimum and maximum intensity (number of worms of positive hosts) for each population and the total of the populations. In total, cranial sinus nematodes were found in 80 porpoises, including 4 hosts in which the worms were highly degraded and unidentifiable. Both *P. sunameri* and *P. asiaeorientalis* occurred in 68 hosts. *Stenurus nanjingensis* was found together with *P. sunameri* and *P. asiaeorientalis* in 2 hosts. The species of the genus *Nasitrema* were found in 62 hosts, including 3 hosts in which the worms were highly degraded and unidentifiable. Both species of this genus were found together in 40 hosts, and coinfection of any trematode and any nematode species occurred in 61 hosts. Lungworms were found in 64 hosts, but were unidentifiable in 29 of these owing to degradation or fragmentation. *H. pingi* and *H. sunameri* were not found in the same host, but *H. taurica* was found with *H. sunameri* in 2 hosts.

Prevalence is shown as percentages in Table 4. The prevalence of all cranial sinus helminths was higher in >110 cm hosts than in <90 cm hosts in all populations. The prevalence of *P. sunameri*, *P. asiaeorientalis* and *N. sunameri* in >110 cm hosts was not significantly different among populations (Fisher's exact test, P>0.05). The prevalence of *N. spathulatum* was significantly

			Total				OB					AT				S	S				IM					TS	
Site	Ir	fected	In	tensity		Infect	ba	Intens	ity	Ini	ected	Int	ensity		Infe	cted	Inte	nsity		Infecte	p	Intens	ity	Inf	ected	Int	ensity
Species		host	mean 1	min. n	laX.	hos	t mea	n min.	max.	_	nost I	nean n	uin. m	laX.	ho	st me	an mi	n. mi	x.	host	mear	n min.	max.	ł	nost I	nean	min. 1
Cranial sinus / Examined host	118				4	-				39					6				11					2			
P. sunameri		75	809	1 5,8	324 ^{a)}	28	903	5	5,824 ^{a)}		26	805	2 3,3	(24 ^{a)}	1	5 75	6 0	1,9(16 ^{a)}	5	421	1	$1,147^{a}$		1	-	,069 ^{a)}
P. asiaeorientalis		69	370	1 2,4	196 ^{a)}	27	417	1 5	$2,496^{a}$		23	239	1 1,3	(80 ^{a)}	1.	5 53	8 5	1,4{	(3a)	б	138	9	235 ^{a)}		1		267 ^{a)}
S. nanjingensis		7	,	1	22	2	'	1	22		0	,			-	- ('	'		0	'	·	ı		0	ı	,
N. sunameri		48	19	1	252	17	7		36		16	33	1	252	-	9 1	1 2	ŝ	ć	5	30	4	55		1	,	17
N. spathulatum		51	15	1	82	19	8	7	31		19	21	1	82	1	3 1	8 1	7	8	0	ı	ı	·		0	ı	,
Lung / Examined host	114				4	2				27				त्य	11				19					2			
H. pingi		22	63	1	302	13	80	5	302		З	7	1	ŝ		'	10	-		2	99	1	130		0	ı	
H. sunameri		13	12	1	58	9	10	-	32		З	13	9	21		3	0 1	Ś	8	1	'	1	,		0	ı	ı
H. taurica		0	,	1	1	1	ı	1	ı		1	ı	1		-	- (I	'		0	ı	·	ı		0	ı	ı
Liver / Examined host	69				6	4				15				1	2				16					7			
C. oblonga		18	30	1	123	1	'	5	ı		7	8	1	30	4	4	1 9	5.	~	5	45	2	123		1	ı	122
Intestine / Examined host	88				3	1				23				-	2				15					2			
S. elongatum		16	13	1	45	З	10	9	12		2	-	2	45		3 1	2	4	0	с	11	1	26		0	,	ı
D. fuhrmanni		б	(q -	[(q -	123	1	'	(q -	ı		0	ı				_	'	- (q		0	ı	ı	ı		1	ı	123
Tetrabothrius sp.		1		1	ı	0	'		ı		1		1		-	C	'	'		0	'	'	·		0	ı	ı
Corynosoma sp.		1	,	426	ı	0	'		ı		0				-	· ('		0	ı	·	ı		1		426

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									Prevalen	ce (%)								
Site		Total			OB			АТ			SIS			IM			TS	
Species	06>	90-110	>110	06>	90-110	>110	06>	90-110	>110	06>	90-110	>110	06>	90-110	>110	06>	90-110	>110
	CIII	CIII	CIII	СШ	CIII	CIII	CIII	CIII	CIII	CIII	сш	CIII	сш	cm	cIII	CIII	cIII	сш
Cranial sinus / Examined host ^{a)}	21	10	50	×	4	15	4	ŝ	15	7	-	14	9	0	S	1	0	1
P. sunameri	10	40	98	25	75	100	0	0	93	0	100	100	0		100	0	·	100
P. asiaeorientalis	14	40	88	25	75	100	0	0	80	50	100	93	0	ı	60	0		100
S. nanjingensis	0	0	4.0	0	0	13	0	0	0	0	0	0	0	·	0	0	·	0
N. sunameri	0	10	70	0	25	67	0	0	67	0	0	64	0	ı	100	0	·	100
N. spathulatum	0	10	72	0	0	80	0	0	80	0	100	86	0	·	0	0		0
Lung / Examined host ^{a)}	34	10	50	11	4	17	4	4	14	4	2	13	14	0	ŝ	1	0	1
H. pingi	35	20	2.0	45	25	0	25	25	7.1	25	0	0	36	ı	0	0		0
H. sunameri	0	10	20	0	25	24	0	0	14	0	0	23	0	ı	20	0		0
H. taurica	0	0	0	0	0	0	0	0	0	0	0	0	0	ı	0	0		0
Liver / Examined host ^{a)}	18	×	32	9	3	14	2	4	8	0	1	S	10	0	S	0	0	0
C. oblonga	0	13	47	0	0	7.1	0	25	63	'	0	80	0	ı	100	ı		ı
Intestine / Examined host ^{a)}	24	13	48	æ	9	15	4	ŝ	14	1	2	13	10	0	3	1	0	1
S. elongatum	4.2	7.7	29	0	17	13	0	0	14	100	0	54	0		60	0	·	0
D. fuhrmanni	0	0	4.2	0	0	0	0	0	0	0	0	7.7	0	·	0	0	,	100
Tetrabothrius sp.	0	0	2.1	0	0	0	0	0	7.1	0	0	0	0		0	0	·	0
Corynosoma sp.	0	0	2.1	0	0	0	0	0	0	0	0	0	0	ı	0	0	ı	100
a) Only hosts which were washed a	nd micr	oscopically	observed ;	are include	ed.													

different between IM and OB, AT and SIS populations (Pairwise Fisher's exact test with Benjamini–Hochberg procedure, adjusted P<0.05). *H. pingi* was frequently found in <90 cm hosts in four of the populations, and its prevalence was not significantly different among populations (Fisher's exact test, P>0.05). In the total of the populations, the prevalence of *H. pingi* in <90 cm hosts was significantly higher than in >110 cm hosts (Fisher's exact test, P<0.01). *H. sunameri* was only found in 90–110 cm and >110 cm hosts in four populations. Its prevalence in >110 cm hosts was not significantly different among populations (Fisher's exact test, P<0.05), but it was significantly higher than in <90 cm hosts in the total of the populations (Fisher's exact test, P<0.05), but it was significantly higher than in <90 cm hosts in the total of the populations (Fisher's exact test, P<0.01). *C. oblonga* was found only in >110 cm hosts, and its prevalence in OB was significantly lower than in AT, SIS and IM (Pairwise Fisher's exact test with Benjamini–Hochberg procedure, adjusted P<0.05). *S. elongatum* was frequently found in >110 cm hosts, and its prevalence was not significantly different among populations (Fisher's exact test, P>0.05).

DISCUSSION

Three species obtained in this study include several synonyms. *Pharurus sunameri* and *Pharurus asiaeorientalis* were originally described as *Pseudostenurus sunameri* Yamaguti, 1951 [38] and *Pharurus asiaeorientalis* Petter, 1982 [27], respectively. The former was included in the genus *Pharurus* [27]; however, the name *Pseudostenurus sunameri* has been used in several later studies [14, 21, 26]. The latter was renamed and transferred to genus *Otophocaenurus* [21]. Both genera (*Pseudostenurus* and *Otophocaenurus*) were considered synonyms of the genus *Pharurus* [3]. The present study follows this classification. *S. elongatum* was originally described as *Orthosplanchnus elongatus* Ozaki, 1935 [24]; subsequently, it was transferred to the genus *Odhneriella* [39] and then to *Hadwenius* [1]. Recently, *Hadwenius* was considered as a synonym of the genus *Synthesium* [11]. Therefore, in this study, a new combination *Synthesium elongatum* n. comb. is used.

A new location record and a new host record were obtained in this study. *S. nanjingensis* was originally described based on specimens obtained from a finless porpoise in Nanjing, China [34]. The detection of this species in the present study is the first record in Japanese waters, although the worms were very rare and were only found in two hosts of the OB population. *Tetrabothrius* sp. was detected for the first time in the genus *Neophocaena*.

This study is the first report about the helminths found in finless porpoises in the OB, AT and IM populations. It also adds further information on the SIS and TS populations, which have been studied previously [21]. Eight species of helminths: *P. sunameri, P. asiaeorientalis, N. spathulatum, N. sunameri, H. pingi, H. sunameri, C. oblonga* and *S. elongatum* were commonly found and are considered common parasitic helminths of finless porpoises in Japan. In marine mammal helminths, anisakid nematodes in the stomach have been reported in several cetaceans [12, 16, 17]. However, no anisakids were found in this study. This is in line with previous findings: another study on the finless porpoises in the SIS and TS populations also found no anisakids [21]. This might be due to the highly coastal habitat of the finless porpoise [14, 19]. On the other hand, Pilleri [28] reported *Anisakis typica* in two hosts of the finless porpoise in Pakistan. Jefferson and Wang [15] proposed that the genus *Neophocaena* includes at least two species: *N. asiaeorientalis* in east Asia and *N. phocaenoides* in the Indian Ocean; both species show extensive geographical variation. Although the prevalence of anisakid infection in *N. phocaenoides* is not reported, Pilleri's report might indicate an inter-species or regional difference in anisakid infection in the genus *Neophocaena*, which probably reflects a difference in local habitat in the genus.

Comparing the helminthic fauna in the different host populations, the species composition or prevalence of helminths was more or less different between each adjacent population. *N. spathulatum* was not found in the IM and TS populations. Although the number of examined hosts from TS was limited, a previous study also found no *N. spathulatum* in the four hosts examined [21]. *N. spathulatum* is considered absent or less prevalent in IM and TS populations than in the other populations. *Corynosoma* sp. was found only in the TS population. Kuramochi *et al.* [21] examined the intestines of three adult finless porpoises from TS and obtained some specimens of the genus *Corynosoma* from all of them. Based on general morphology, these worms were regarded as the same species. This acanthocephalan species is considered specific to TS. The prevalence of *C. oblonga* in the OB population was lower than in the other populations, with the exception of TS. The only available information on *C. oblonga* infection in the TS population is from Kuramochi *et al.* [21]. In their study, one of three examined adult porpoises was infected. If the data from the present study and Kuramochi *et al.* [21] are combined, the best estimate of the prevalence of *C. oblonga* in the TS population increases to 50% (2/4), higher than the OB population.

Not only for the finless porpoise, information on the lifecycles of marine mammal helminths is generally very scarce. *C. oblonga* has been found in the harbor porpoise (*Phocoena phocoena*) [7, 12, 32, 33], Dall's porpoise (*Phocaenoides dalli*) [6] and the common dolphin (*Delphinus delphis*) [12] from a wide range of habitats in the northern hemisphere. An atypical-host infection has also been reported in the common thresher shark (*Alopias vulpinus*) [2]. Based on the feeding habits of these hosts, the transmission of *C. oblonga* is suspected to involve the consumption of fish. On the other hand, *N. spathulatum* has only been found in finless porpoises in Japan, and there is no information available about its lifecycle. In this study, *N. spathulatum* was found exclusively in porpoises that were longer than 90 cm, which were designated weaning or weaned (see Materials and Methods). Therefore, one or multiple prey species might serve as intermediate/paratenic hosts. From stomach content analyses, the finless porpoise is known to be an opportunistic feeder, which feeds on several fishes, cephalopods, crustaceans and other marine organisms [20, 30, 31]. The differences in the presence and prevalence of helminths found in this study might reflect differences in the available prey species in each population.

The prevalence of *H. pingi* was higher in <90 cm porpoises, which were considered nursing (see Materials and Methods), than in >110 cm porpoises, considered weaned. The preferential infection by *H. pingi* of neonate finless porpoises (the exact species are

unknown) has been reported in Hong Kong, and prenatal or transmammary infection has also been suspected [25, 26]. The present study also supports this possibility. Such vertical transmission has also been suspected during H. lagenorhynchi infection of the bottlenose dolphin (Tursiops truncatus) [8, 10] and the common dolphin (D. delphis) [35]. If the vertical transmission hypothesis is true, it is strange that *H. pingi* is almost not found in >110 cm porpoises. If the worms are transmitted from the mother to the fetus or the neonate, the prevalence of infected adults should be equal to or higher than that of calves. The pathogenicity of H. pingi could increase the fatality of severely infected neonates, which would result in high numbers of young animals infected by *H. pingi* being stranded. Thus, the prevalence of *H. pingi* in <90 cm porpoises might appear higher than in >110 cm porpoises. On the other hand, a congeneric species H. sunameri was found frequently in >110 cm porpoises, but not found in <90 cm porpoises, indicating that H. sunameri infects porpoises via infected prey. In lungworms found in the harbor porpoise, a correlation between the host age and the number of species of lungworms has been observed [5], and a heteroxenous lifecycle, i.e., the existence of intermediate host species, has been suggested [22]. Although the species reported in the harbor porpoise are different from those in the finless porpoise [4, 9, 12], a heteroxenous lifecycle of finless porpoise lungworms is plausible. The presence of completely different infection routes between the two congenerics (the vertical transmission of *H. pingi* and the trans-prey infection of *H. sunameri*) is very interesting. Such shifts in lungworm species with the host's growth have not been reported in other cetaceans. To verify the infection routes, detection of *H. pingi* in fetuses or milk, and the identification of infective *H. sunameri* larvae from prey species are needed.

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