Primer	Sequence (5'-3')
MiFish U-F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNGTCGGTAAAACTCGTGCCAGC
MiFish Ev2-F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNRGTTGGTAAATCTCGTGCCAGC
MiFish U2-F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNGCCGGTAAAACTCGTGCCAGC
MiFish U-R	GTCTCGTGGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNCATAGTGGGGTATCTAATCCCAGTTTG
MiFish Ev2-R	GTCTCGTGGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNGCATAGTGGGGTATCTAATCCTAGTTTG
MiFish U2-R	GTCTCGTGGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNCATAGGAGGGTGTCTAATCCCCGTTTG

Table S1. List of MiFish primers used with the Nextera XT index kit (Illumina, San Diego, California, USA)

Table S2. List of fish species examined in this study, showing their ecological characteristics and total abundances in the eelgrass meadows based on analysis of eDNA collected within (IN) and above (OUT) eelgrass meadows and by sledge-net sampling (SN). For eDNA metabarcoding, total read abundances are reported. Swimming position (SP) includes bottom swimmers (BS) and surface swimmers (SS), and appearance frequency in seagrass habitats (Freq.) includes year-round residents (YR), seasonal residents (SR), transient species (T), and casual species (C). IRS indicates that we listed characteristics that are common among related species

Total abundance										
FAMILY	SPECIES	Ka	sado Bay	7	Kuri	hama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
ANGUILLIFOR	MES									
Anguillidae	Anguilla japonica		168			309		BS	С	3, 5
Congridae	Conger myriaster	72						BS	Т	3, 13, 19
Muraenesocidae	Muraenesox cinereus	27	56					BS	С	3
Ophichthidae	Ophisurus macrorhynchos				53	125		BS	С	3, 6
	Ophichthus altipennis	248				55		BS	Т	1, 3, 6, 9, 16
ATHERINIFOR	MES									
Atherinidae	Hypoatherina valenciennei	28,881	7,588		622	1,219		SS	Т	3, 8, 9, 12, 16
	Hypoatherina woodwardi		1,349					SS	С	3
AULOPIFORMI	ES									
Synodontidae	Saurida elongata	29	21					BS	С	3

Total abundance										
FAMILY	SPECIES	K	asado Ba	ıy	Kur	ihama Ba	ıy	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
BELONIFORMES										
Belonidae	Strongylura anastomella		2,375					SS	С	3
Exocoetidae	Cheilopogon sp.					211		SS	С	3 (IRS)
	Cypselurus sp.		25					SS	С	3 (IRS)
Hemiramphidae	Hyporhamphus sajori	907	11,089			1,253		SS	Т	3, 8, 11, 12, 14, 16
CLUPEIFORM	ΞS									
Clupeidae	Konosirus punctatus	16,838	14,897		54,008	32,472		SS	С	3, 14
	Sardinella zunasi		66		177	95		SS	Т	3, 8, 11, 12
	Sardinops sagax				3,255	2,169		SS	С	3
	Spratelloides gracilis				248	406		SS	SR	3, 9, 12
Dussumieriidae	Etrumeus teres				368	98		SS	Т	3, 9, 12, 12, 15
Engraulidae	Engraulis japonicus	19,574	55,914		9,312	12,518		SS	Т	3, 9, 11, 12
GASTEROSTEI	FORMES									
Hypoptychidae	Aulichthys japonicus				183		4	BS	YR	3, 6, 8, 11, 19
MUGILIFORM	ES (Mugiloidei)									
Mugilidae	Mugil cephalus	22,081	19,021		85,847	152,446		BS	SR	1, 3, 5, 6, 8, 10, 11, 13, 19
	Planiliza haematocheila		52					SS	С	3

			То	tal ab	undance					
FAMILY	SPECIES	Kas	sado Bay	7	Kuri	ihama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
PERCIFORMES	8									
ACANTHUROID	DEI									
Siganidae	Siganus fuscescens	386	81		228	292		BS	SR	1, 3, 9, 10, 12, 13, 16, 18
BLENNIOIDEI										
Blenniidae	Omobranchus elegans		15			17		BS	С	3
	Parablennius yatabei		13		67			BS	С	1, 3, 13
Chaenopsidae	Neoclinus bryope		57			57		BS	YR	3, 19
Tripterygiidae	Enneapterygius etheostomus				192			BS	С	3
CALLIONYMOI	DEI									
Callionymidae	Callionymus valenciennei				3,614	502		BS	SR	3, 11, 18
	Callionymus japonicus or Callionymus curvicornis				679	1,014		BS	SR	1, 3, 6, 10, 11
	Repomucenus ornatipinnis or Callionymus beniteguri	222			1,099	3,488		BS	YR	3, 6, 8, 9, 10, 11, 13, 18
GOBIOIDEI										
Gobiidae	Acanthogobius flavimanus				1,179	1180		BS	YR	3, 5, 6, 9, 12, 16, 18
	Acentrogobius pflaumii	2,339	1137	1	22,825	14,564	24	BS	YR	3, 4, 5, 6, 8, 9, 10, 13, 18, 20
	Amblychaeturichthys sciistius				238			BS	С	1, 3

			То	tal abı	undance					
FAMILY	SPECIES	Ka	sado Bay	7	Kuri	hama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
	Callogobius tanegasimae				284	26		BS	С	3
	Eviota abax					74		BS	С	3
	Favonigobius gymnauchen	208	431		1,085	5,246	3	BS	YR	1, 3, 5, 6, 8, 9, 10, 11, 12, 18, 20
	Glossogobius olivaceus				51			BS	С	3
	Gymnogobius breunigii		363					BS	Т	3, 12, 18
	Gymnogobius heptacanthus	1,191				152		BS	YR	3, 4, 6, 11, 12, 18
	Gymnogobius urotaenia				216	229		BS	Т	3, 12
	Istigobius campbelli				476	28		BS	С	1, 3
	Istigobius sp.					17		BS	С	3
	Istigobius hoshinonis				470	195		BS	С	3
	Leucopsarion petersii or Priolepis boreus				175			BS	С	3, 18
	Myersina filifer		201		307			BS	С	3
	Mugilogobius abei				111	112		BS	С	3
	Redigobius bikolanus				717	380		BS	С	3
	Rhinogobius sp.1 (R. cf. fluviatilis)				142	717		BS	С	3 (IRS)
	Rhinogobius sp.2		239					BS	С	3 (IRS)

			То	tal abı	undance					
FAMILY	SPECIES	Kas	sado Bay	7	Kuri	hama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
	Pterogobius elapoides					223		BS	YR	3, 4, 6, 8, 9, 11, 19
	Pterogobius zonoleucus				677	431	7	BS	YR	1, 3, 6, 9, 19, 20
	Sagamia geneionema				7,697	6,720		BS	YR	1, 3, 6, 9, 13, 14, 19
	Taenioides sp. (T. cf. cirratus)		13					BS	SR	2, 3
	Tridentiger spp. (T. obscurus, T. brevispinis)		15		1,515	1,710		BS	С	3
	Tridentiger trigonocephalus	92			2,727	1,889		BS	YR	3, 8, 11, 12, 13, 16, 18
Microdesmidae LABROIDEI	Parioglossus dotui					227		BS	С	3
Embiotocidae	Ditrema spp. (D. jordani, D. temminckii pacificum, D. temmincki temmincki, D. viride)	153,605	7,493		57,347	50,536	1	BS	YR	3, 6, 8, 9, 10, 11, 12, 18, 19, 20
	Neoditrema ransonnetii					373		BS	С	3
Labridae	Halichoeres tenuispinis		479		868	467		BS	YR	3, 8, 9, 10, 11, 13, 19
	Parajulis poecilepterus	1,249	6		25,029	3,420		BS	YR	3, 6, 8, 9, 10, 11, 13, 19
	Semicossyphus reticulatus					15		BS	С	3, 19
	Suezichthys gracilis				487			BS	С	3, 13, 19

			То	tal abu	undance					
FAMILY	SPECIES	Kas	sado Bay	y	Kuri	hama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
PERCOIDEI										
Apogonidae	Apogonichthyoides niger						2	BS	YR	1, 3, 13, 20
	Jaydia lineata					6		BS	Т	3, 13 (IRS)
Carangidae	Decapterus maruadsi					7		SS	Т	3, 8, 14
	Seriola quinqueradiata				1,345	202		SS	С	3, 13
	Trachurus japonicus	923			22,082	8,805		SS	Т	1, 3, 9, 11, 12, 13, 19
Cheilodactylidae	Cheilodactylus sp. (C. cf. zonatus)				311	206		BS	SR	3, 6, 9, 12, 13
Haemulidae	Plectorhinchus cinctus	13				99		BS	С	3, 10, 13, 18
	Parapristipoma trilineatum				5,569	6,469		BS	SR	3, 9, 13, 19
Kyphosidae	Girella punctata	2,082	75		4,257	2,755		BS	SR	1, 3, 11, 12, 13, 16, 19
Lateolabracidae	Lateolabrax japonicus	8,072	1,093		6,787	2,995		BS	SR	3, 8, 10, 11, 12, 15, 16, 18
	Lateolabrax latus				9			BS	С	3
Leiognathidae	Equulites rivulatus				2,148	1,539		BS	С	3, 8
	Nuchequula nuchalis				3,268	1,994		SS	Т	3, 9, 12, 18
Mullidae	Upeneus japonicus					176		BS	SR	1, 3, 6, 9, 13
Oplegnathidae	Oplegnathus fasciatus				113	16		BS	С	3, 6, 9, 13

		Total abundance								
FAMILY	SPECIES	Kas	sado Bay	7	Kuri	hama Bay	7	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
Sciaenidae	Pennahia argentata		4		1,454	83		BS	С	3
Scombropidae	Scombrops spp. (S. boops, S. gilberti)				253	65		BS	SR	1, 3, 6, 9, 12, 13, 19
Serranidae	Epinephelus akaara	585	74					BS	С	3
Sillaginidae	Sillago japonica	5,336	32		11,928	14,330		BS	SR	3, 6, 8, 9, 11, 12, 13, 16, 18, 19
Sparidae	Acanthopagrus latus	462	179		1,649	4383		BS	Т	3, 8, 13, 16, 18
	Acanthopagrus schlegelii	42,076	57,292		100,425	133,259		BS	SR	1, 3, 6, 8, 11, 12, 13, 14, 16, 18, 19
	Pagrus major	250	17		8,621	594		BS	SR	1, 3, 8, 9, 11, 13, 15
	Rhabdosargus sarba				619			BS	SR	3, 12, 16
Terapontidae	Rhynchopelates oxyrhynchus				247	455		BS	SR	3, 9, 12, 16
SCOMBROIDEI										
Scombridae	Scomber spp. (S. japonicus, S. australasicus)	3,035	597		7,892	1,834		SS	С	3, 13
	Trichiurus lepturus				61			BS	С	3
Sphyraenidae	Sphyraena japonica				654	1,121		SS	С	3, 12
	Sphyraena pinguis	148	329		2,747	1,374		SS	SR	3, 10, 13
TRACHINOIDEI										
Pinguipedidae	Parapercis pulchella					9		BS	С	3, 9
	Parapercis snyderi				42			BS	YR	1, 3, 13

	Total abundance									
FAMILY	SPECIES	Kas	ado Bay	7	Kuri	hama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
ZOARCOIDEI										
Pholidae	Pholis crassispina	1,265						BS	SR	3, 17
	Pholis nebulosa				282	805		BS	SR	3, 5, 8, 9, 10, 12, 13, 15, 18
Stichaeidae	Dictyosoma burgeri (Form a)				1,182	362		BS	С	3
	Dictyosoma rubrimaculatum				258	96		BS	С	3
	Zoarchias neglectus				517	704		BS	С	3
PLEURONECTI	FORMES									
Cynoglossidae	Cynoglossus interruptus				34			BS	С	3
	Cynoglossus joyneri				76			BS	С	3
	Cynoglossus robustus	53	15					BS	С	3
	Paraplagusia japonica				840	3,124		BS	С	3, 12
Paralichthyidae	Paralichthys olivaceus		42		2,406	946		BS	С	3
Pleuronectidae	Platichthys bicoloratus	169						BS	SR	3, 10
	Pseudopleuronectes yokohamae	5,372	58		412	638		BS	SR	3, 5, 6, 10, 12, 18
	Verasper variegatus	191						BS	С	3
Soleidae	Heteromycteris japonicus				139	69	1	BS	SR	3, 10, 20
	Pseudaesopia japonica					38		BS	Т	3, 7, 8

		Total abundance									
FAMILY	SPECIES	Kas	ado Bay	r	Kuri	hama Ba	ay	SP	Freq.	Reference	
		IN	OUT	SN	IN	OUT	SN				
SCORPAENIFO	RMES										
COTTOIDEI											
Cottidae	Pseudoblennius cottoides	165,481	71926	1			5	BS	YR	3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 18, 20	
	Pseudoblennius percoides	57,228			861	1,550	4	BS	YR	3, 6, 9, 10, 12, 13, 18, 20	
	Pseudoblennis sp.3 (KIRINANAHAZE)	955,311						BS	SR	3 (IRS), 16	
HEXAGRAMMO	IDEI										
Hexagrammidae	Hexagrammos otakii	8,544	17,045		288	57		BS	SR	3, 8, 11, 15, 16	
	Hexagrammos agrammus	2,826	503			130		BS	SR	3, 6, 8, 9, 10, 11, 13, 15, 16, 18	
PLATYCEPHALO	DIDEI										
Platycephalidae	Onigocia spinosa							BS	С	3, 9	
	Platycephalus spp.		1,234		1,304	877		BS	С	1, 3 (IRS), 18	
SCORPAENOIDE	EI										
Scorpaenidae	Scorpaena miostoma					133		BS	С	3	
	Scorpaenodes evides				1,291			BS	С	3,9	

Total abundance										
FAMILY	SPECIES	Kas	sado Bay	7	Kuri	hama Ba	ay	SP	Freq.	Reference
		IN	OUT	SN	IN	OUT	SN			
Sebastidae	Sebastes oblongus	2,865						BS	С	3, 11, 15
	Sebastes sp.1 (S. cf. cheni)	41,575	4,699	35	4,116	7,426	7	BS	SR	3 (IRS), 8, 10, 11, 12, 18, 20
	Sebastes sp.2 (S. cf. inermis)			6		280	1	BS	SR	1, 3, 5, 6, 9, 11, 12, 15, 13, 18, 20
	Sebastiscus marmoratus	455	405		2030	968		BS	YR	1, 3, 8, 9, 11, 16
Synanceiidae	Inimicus japonicus	45	51					BS	С	3, 8, 11
Tetrarogidae	Paracentropogon rubripinnis	580		7	14,431	17,748	212	BS	YR	1, 3, 6, 8, 9, 10, 11, 12, 13, 18, 20
SILURIFORME	S									
Plotosidae	Plotosus japonicus	102			671	49	2	BS	YR	3, 9, 10, 11, 12, 13, 15, 16, 18, 20
SYNGNATHIFO	ORMES									
Syngnathidae	Hippocampus coronatus			4			10	BS	YR	3, 5, 8, 9, 11, 12, 18, 20
	Syngnathus schlegeli	109		5		441	10	BS	YR	1, 3, 6, 8, 9, 10, 11, 12, 13, 18, 20
	Urocampus nanus	100			27	274		BS	YR	3, 8, 9, 10, 11, 12, 16, 18
TETRAODONT	IFORMES									
Monacanthidae	Cantherhines pardalis					198		BS	С	3
	Rudarius ercodes	3,768	148	5	3,887	2,640	21	BS	YR	1, 3, 5, 6, 8, 9, 10, 11, 12, 13, 16, 18, 20
	Stephanolepis cirrhifer	169	44		346	148		BS	SR	1, 3, 8, 9, 10, 11, 12, 13
	Thamnaconus modestus	5,714	5					BS	SR	1, 3, 8, 9, 11, 13

	_		To	tal abu	Indance					Reference
FAMILY	SPECIES	Kas	sado Bay	•	Kuri	hama Ba	ny	SP	Freq.	
		IN	OUT	SN	IN	OUT	SN			
Ostraciidae	Ostracion immaculatus				2,988	544		BS	SR	1, 3, 9, 13, 19
Tetraodontidae	Takifugu spp.1 (T. niphobles, T. pardalis, T. poecilonotus)	47,252	6372		47,332	39,366	2	BS	YR	1, 3, 6, 8, 9, 10, 11, 12, 15, 16, 18, 19, 20
	Takifugu spp.2 (<i>T. stictonotus</i> , <i>T. snyderi</i>)				31	99		BS	С	3, 9, 15
Triacanthidae	Triacanthus biaculeatus				124			BS	Т	3, 9, 12, 15
MYLIOBATIFO	PRMES									
Dasyatidae	Hemitrygon akajei	868	114		5			BS	С	3, 9, 12
Gymnuridae	Gymnura japonica	4						BS	С	3
Myliobatidae	Aetobatus narutobiei		89					BS	С	3
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ID	SOURCE									
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ID	SOURCE
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Fig. S1. The number of species detected per sampling day for eDNA collected from within eelgrass meadows (IN; n = 4 per sampling date), above eelgrass meadows (OUT; n = 4), and from sledge-net sampling (SN; n = 1) at (a) Kasado Bay and (b) Kurihama Bay. For IN and OUT, error bars represent one standard deviation. Paler colors indicate samples collected earlier in the study



Fig. S2. The number of species identified per ecological characteristic in Kasado Bay (black) and Kurihama Bay (white). Bottom, bottom swimmer; Surface, surface swimmer; YR, year-round resident; SR, seasonal resident; T, transient species; C, casual species (see Table 2 for details)



Fig. S3. Species accumulation curves for eDNA_{in} (green; eDNA collected from within eelgrass meadows), eDNA_{out} (blue; eDNA collected from above eelgrass meadows), and sledge-net sampling (yellow) for (a) Kasado Bay and (b) Kurihama Bay. Shaded areas represent 95% confidence intervals. For sledge-net sampling, the species accumulation curves have been extrapolated to 4–6 samples (dashed lines)