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1	Tracking the Northern Pacific sea star Asterias amurensis with
2	acoustic transmitters in the scallop mariculture field of Hokkaido,
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Abstract

The Northern Pacific sea star Asterias amurensis has a major negative impact on scallop mariculture. In northern Japan, fishermen clean up sea stars before releasing young scallops in the mariculture field; however, new sea stars constantly invade the field from outside areas to predate on scallops. Thus, it is important to determine the migration speed and seasonal behavioral patterns of the Northern Pacific sea star to implement effective density control measures. Here, we set out to quantify these parameters using acoustic telemetry. In a rearing experiment, acoustic transmitters were retained on sea stars for up to 71 days using nylon fishing line. In the field experiment, we showed that the moving distance of the Northern Pacific sea star over a one-week period was significantly further in spring $(90.9 \pm 49.9 \text{ m})$ than in summer $(25.1 \pm 18.9 \text{ m})$, and that the moving speed was significantly faster in spring $(18.1 \pm 15.2 \text{ m/day})$ than in summer $(4.3 \pm 9.1 \text{ m/day})$. Our results are the first to present the two-dimensional movement of Northern Pacific sea star individuals in spring and summer. We suggest that sea star extermination practices should be extended beyond the immediate culture area.

Keywords: acoustic telemetry; tag attachment; behavior; Northern Pacific sea star

1 Introduction

17

 $\mathbf{2}$ Sea stars Asterias sp. intensively prey on bivalves, negatively impacting the aquaculture of commercially important bivalves globally (Byrne et al. 2013). Therefore, 3 4 it is important to understand the motility of sea stars to quantify the potential predation impact of sea stars on cultured bivalves, and, hence, the efficiency of various practices, $\mathbf{5}$ 6 such as extermination. In Japan, North America, and Australia, sea stars are regularly $\overline{7}$ exterminated in grounds where bivalves are cultured. Freeman et al. (2001) and Gallagher et al. (2008) indirectly estimated the migration distance of sea stars based on seasonal 8 9 changes in dense areas. However, the migration ability and seasonal migratory range of 10 individual sea stars remains unclarified. 11 Recent advances in electronic technologies have enabled scientists to develop 12sophisticated biotelemetry methods to monitor the location, behavior, and physiology of free-ranging marine animals (Cooke et al. 2004). Biotelemetry provides a useful method 1314to monitor and collect data on the biology of animals that are not easily detectable, while 15minimally influencing an animal's behavior. This approach allows the collection of more data than is possible by other techniques, such as mark and recapture. It also allows 16

18 A method has already been developed to attach underwater acoustic transmitters to

physiological and behavioral data collected in the laboratory and the field to be compared.

19 *Coscinasterias muricata* and *Protoreaster nodosus* sea stars, which are widely distributed
20 in the sub-tropical and tropical zone, facilitating the direct analysis of their motility and
21 behavior (Lamare et al. 2009; Chim and Tan 2013).

22The mariculture of Japanese scallops *Mizuhopecten vessoensis* has prospered on the eastern coast of Hokkaido Island, Japan, and has strong similarities with agriculture. The 2324fishing grounds are separated into three or four sections, with young scallops (c.a. 1 year old) being released with a one-year delay in each section (Chiba and Arai 2014). After 25three to four years, the mature scallops are harvested by a dredge net, after which more 2627young scallops are released in the emptied section again. Scallop mariculture is a very 28important fishery in east Hokkaido. However, the scallops are exposed to predation by 29sea stars, which has led to major economic issues (Imai 1978; Chiba and Arai 2014). 30 Therefore, fisherman clean up sea stars in most scallop mariculture fields with dredge nets before releasing young scallops. This action reduces the density of Northern Pacific 31sea star Asterias amurensis individuals to nearly zero in these areas. However, new sea 32stars constantly invade the mariculture zone from outside areas to predate on scallops. 33 Therefore, it is important to gather direct information on the migration speed and 3435seasonal behavioral patterns of Northern Pacific sea stars to implement effective density control measures. This study aimed to (1) develop a method of installing acoustic 36

37	transmitters on Northern Pacific sea stars, and (2) investigate the motility and behavior
38	of sea stars in the scallop mariculture field using acoustic telemetry. Our results are
39	expected to inform managers on the extent to which extermination practices should be
40	conducted beyond the immediate culture area.
41	
42	Materials and methods
43	Rearing experiment
44	In April and July of 2013, we conducted a rearing experiment to design an adequate
45	method of installing acoustic transmitters on Northern Pacific sea stars. The sea stars (n
46	= 47) were collected by towing a dredge net on the sea bottom (c.a. $35-50$ m depth areas)
47	of the Sea of Okhotsk off Abashiri Bay (Fig. 1a). Collected sea stars were transferred to
48	a compact water tank (L \times W \times H = 1.8 \times 0.9 \times 0.7 m) set in the Abashiri City Fisheries
49	Science Center. Natural sea water was pumped into each tank at about 0.7 l/h. Water
50	temperature ranged from 1.8 to 17.8 °C.
51	Acoustic transmitters (model V9-1H, VEMCO Ltd., Halifax, Nova Scotia, Canada;
52	3.6 g in air, 9 mm in diameter, and 24 mm in length) were attached externally to the body
53	surface of sea stars ($n = 28$, Table 1). Transmitters were fixed on the upper surface of the
54	central part of a given arm with nylon fishing line (diameter $= 0.37$ mm) using the same

 $\mathbf{5}$

55	procedure developed for the knobby sea star (Chim and Tan 2013). The nylon fishing line
56	was fixed on the arm from the central part of the upper surface to the inside ambulacral
57	groove with small stainless-steel needles (diameter = 0.97 mm). Subsequently, the end of
58	the line was fixed in the opposite direction. Thereafter, the transmitter was tied to the
59	upper surface using the ends of the line. The knot of the line was sealed using two-
60	component epoxy resin. This surgery was carried out twice per specimen; consequently,
61	both ends of the transmitter were fixed to the surface of each sea star using nylon lines. A
62	total of 14–22 juvenile scallops (shell length 4.9 \pm 0.6 cm, weight 18.2 \pm 5.4 g) were
63	placed in each tank as prey for sea stars. When dead scallops were found, new scallops
64	were added to maintain the initial density of scallops for about once a week during the
65	rearing experiments.
66	
67	Tracking study in the field
68	Study area
69	We conducted the field study in Nemuro Bay, which is located on the east side of
70	Hokkaido (Fig 1). Most of Nemuro Bay is shallower than 20 m, with a profitable scallop-
71	mariculture field covering a wide area of this bay. We set square study sites of 500×500
72	m in 2013, and 450 \times 450 m in 2014 in an area of c.a. 2 km from the shoreline (depth

73	ranges: 5-12 m). At each corner (named as Stns. 1-4), a monitoring receiver (VR2W,
74	Vemco Ltd.) was moored from July 23 to October 30, 2013, and from May 21 to July 25,
75	2014. The bottom water temperature and velocity conditions in the study areas were
76	monitored using data loggers (Logger version 2-D electro-magnetic current meter
77	INFINITY-EM AEM-USB, JFE Advantech Co., Ltd.) deployed 1 m above the bottom of
78	Stn. 5 (Fig 1b).

79 Acoustic telemetry and experimental animals

The position and movement of sea stars underwater were estimated using acoustic 80 81 telemetry. For the VEMCO system, the method called VEMCO Positioning System 82 (VPS) is available. For a detailed description on the methods used for VPS analysis, see Espinoza et al. (Espinoza et al. 2011) and Smith (http://vemco.com/wp-83 content/uploads/2013/09/understanding-hpe-vps.pdf/ "Accessed 1 April 2017"). Before 84 starting the animal tracking experiment, we estimated the horizontal position errors (HPE) 85 when estimating the location of transmitters using a method that is exclusive to the 86 87 VEMCO system. In our field site, the HPE of 90% of transmitter data positions were within 6.3 m in 2013 and 6.4 m in 2014. 88

Sea stars were captured using a dredge net in the marine area near to our study site,
and were transferred to outdoor tanks at Odaitoh Fishing Port, where we installed the

91	transmitters (Fig. 1b). Acoustic transmitters (model V9-1H, 69 kHz, VEMCO Ltd.,
92	average pulse rate of 80 sec, pulse range 45-135 sec, expected battery life of 80 days)
93	were immediately installed on seven and 11 sea stars in 2013 and 2014, respectively.
94	A dummy individual with no active movement was released in the same area to
95	compare the rate of transmissions with live (moving) sea stars and to confirm that sea star
96	movement was not just drift caused by sea currents. A dummy scallop (with the innards
97	removed and sinkers added) also had a transmitter installed on the outside of the shell
98	each year, respectively (shell length: 11.8 cm, wet weight 249.1 g in 2013, shell length:
99	12.6 cm, wet weight 236.0 g in 2014).
100	All specimens were reared in a water tank for c.a. 20 h. Before releasing the
101	specimens, the diver removed all benthos (e.g., sea stars) from the central area of the
102	study sites (around Stn. 5) and released the specimens every 10 m. Thereafter, divers
103	released each sea star and scallop with attached transmitters in the center of the circles.
104	
105	Statistical analyses
106	All data processing and statistical analyses were performed using the R statistical
107	computing package (hereafter "R"; Version 3.2.0 Development Core Team, 2015). For
108	the rearing experiments, the retention rate of transmitters on each day was estimated by

the Kaplan-Meier survival estimate. Before and after the rearing experiments, the length
and weight of starfish rays were measured, and the increment of both measurements was
used to calculate the growth rate during the experiments. Thereafter, each growth rate was
compared between tagged and non-tagged individuals by a Student's *t* test.

For the field study, we recorded the positions of each transmitter every 30 min. When 113 114 using VPS telemetry, detecting the location of specimen requires that at least three receivers receive clear signals from each transmitter at the same time. If the signal is 115116 received by fewer than three receivers, data positions are not collected, and the interval 117of detecting the location of each specimen exceeds 30 min. After starting our experiments, 118 the frequency of tracking location intervals increased from >30 min intervals to about 119 two-week intervals. This study was designed to examine detailed and individual-based 120movement of sea stars. Thus, long time intervals between each tracking location were not adequate for our study. Therefore, when the regularity of the tracking rate (i.e., the 121122frequency of 30 min detection/that of total detection from the onset of the experiments) of the dummies fell below 90%, we stopped recording the locations of all specimens 123(programmed period). 124

125 The total moving distance of each specimen was defined as the straight distance of 126 two geographical positions (i.e., the positions in which each specimen existed at the start

127	and the farthest point during the programed period). We analyzed the specific movement
128	behavior of both species in the context of (1) detection of active movement, (2) directional
129	trend in movement, and (3) moving speed during the programed period.

131 (1) Detection of active movement

132Monotonous upward or downward trends in movement from each subsequent 133position were analyzed by the Mann-Kendall test. Significant upward or downward trends 134on the x axis indicate monotonous eastward or westward movements. Significant upward 135or downward trends on the y axis indicate monotonous northward or southward movement. If significant monotonous movement was not detected by the Mann-Kendall 136test on either axis, we compared the moving distance of the dummies and those of the 137138specimens using Spearman correlation coefficients. If there was significant correlation between the dummies and these specimens, we defined them as individuals that did not 139140 show active movement (i.e., the movement of these specimens was almost passive).

141

142 (2) Directional trend in movement

The Rayleigh test is a classic angular analysis, in which the relationship of the
position and direction of each individual is estimated (Zar 1999). The specific directional

145 trends the movement from each successive tracked location was analyzed.

146

147	(3) Movement speed
148	The movement speed was estimated from moving distance of each specimen per unit
149	time (hourly or daily) during the programed period. The moving speed was compared
150	between 2013 and 2014 with a Mann-Whitney U test. The 95% confidence interval of
151	the mean movement speed was calculated by expanding the obtained data set using the
152	boot strap method (Basic method), which was repeated 1000 times.
153	
154	Results
155	Rearing experiment
156	Acoustic transmitters were retained for 71 days on the Northern Pacific sea stars.
157	The retention rate of the transmitters on each day decreased by 20% for 73 days and by
158	50% for 90 days (Fig. 2). There was no significant difference in the growth rates (based
159	on ray length and wet weight) of tagged and untagged starfish after the experiment ($p >$
160	0.05; Table 1).
161	

162 Tracking study in the field

163	In 2013 and 2014, a total of 789,434 and 29,773 locations were detected for sea stars,
164	respectively. The regularity of the tracking rate of the dummies fell below 90% on July
165	31 in 2013 and May 31 in 2014. Further migration analyses were conducted from July 23
166	to July 30 in 2013, and from May 23 to May 30 in 2014 (8 days; programmed period). A
167	total of 6,226 and 10,645 sea star locations were recorded during the programed period
168	in 2013 and 2014, respectively.
169	Mean water temperatures (\pm SD) in the experimental and programed periods in 2013
170	were 16.3 \pm 1.7 °C (range: 12.3–18.9 °C) and 14.2 \pm 0.3 °C (range: 13.3–14.8 °C),
171	respectively. In 2014, these temperatures were 8.5 \pm 2.0 °C (range: 4.7–12.0 °C) and 5.8
172	±0.5 °C (range: 4.8–6.8 °C), respectively. Mean water velocities on the sea bottom in the
173	experimental and programed periods in 2013 were 8.8 ± 4.6 cm/s (range: 0.6–28.9 cm/s)
174	and 17.1 \pm 3.5 cm/s (range: 5.2–26.9 cm/s), respectively. In 2014, these velocities were
175	4.0 ± 3.4 cm/s (range: 0.8–20.7) and 8.3 \pm 3.5 cm/s (range: 3.4–20.7 cm/s), respectively.
176	During the programed periods, the mean (\pm SE) total moving distance of Northern
177	Pacific sea stars was 25.1 \pm 18.9 m in 2013 and 90.9 \pm 49.9 m in 2014 (Fig. 3, Table 2).
178	There was a temporal trend in movement patterns on either the x or y axis for all sea stars
179	in 2013 and 2014 (Table 3).

180 In 2013, there was no particular directional movement by six sea stars (Rayleigh test,

181	p > 0.05; Table 3); however, one other individual (#6) significantly moved in a
182	northeasterly direction ($p < 0.05$). In 2014, seven sea stars showed significant directional
183	movement ($p < 0.05$). Four of those individuals (# 10, 11, 12, and 15) tended to move in
184	a northeastern direction and other three individuals (# 16, 17, and 18) tended to move in
185	a northwestern direction. The direction of the dummies was random in each year.
186	Mean (\pm SE) hourly moving speeds of Northern Pacific sea stars in 2013 and 2014
187	were 1.3 ± 0.1 and 2.2 ± 0.1 m/h. Hourly moving speeds ranged from 0.1 to 16.3 m/h in
188	2013 and from 0.1 to 45.9 m/h in 2014 ($p > 0.05$; Table 3). The mean (+ SE) daily moving
189	speeds of Northern Pacific sea stars in 2013 and 2014 were 4.3 \pm 9.1 and 18.1 \pm 15.2
190	m/day, respectively. In 2014, the daily moving speed was significantly faster than in 2013
191	(p < 0.01). The 95% bootstrap confidence intervals for the moving speed of sea stars in
192	2013 and 2014 were 3.2–14.6 and 17.2–29.9 m/day, respectively.
193	
194	Discussion
195	Rearing experiment
196	In general, sea stars are sensitive to the external stimulation of their internal body

bodies (Olsen et al. 2015). In our study, acoustic transmitters were retained for 71 days. 198

197

13

parts, and have an extraordinary capacity to remove substances that intrude into their

199	The retention rate of transmitters decreased by 20% at 73 days and by 50% at 90 days.
200	There was extensive autotomy and cannibalism after 71 days. The growth rate did not
201	significantly differ between tagged and untagged sea stars; thus, the installation of
202	transmitters probably did not affect the vitals of Northern Pacific sea stars until 71 days.
203	Chim and Tan (2013) installed acoustic transmitters on the knobby sea star Protoreaster
204	nodosus using the same procedures as in our study, with tags being retained for an average
205	of 60 days, and only a few individuals exhibiting extensive damage to their bodies (<10%
206	of all inds.). Our results combined with this previous study show that the installation of
207	transmitters to the ambulacral groove is probably a suitable method for sea stars.
208	Olsen et al. (2015) found that Asterias rubens retained acoustic transmitters injected
209	on the inside of the body wall for over 200 h when in a thermal environment of 10 $^{\circ}$ C;
210	however, the acoustic transmitters were lost within 3 h when the sea stars were in a
211	thermal environment of 16 °C. Therefore, higher temperatures might lead to the faster
212	egestion of invaded materials, as part of the physiology and physics of ectothermic
213	echinoderms. Water temperature during our experiment was 1.8-17.8 °C, with acoustic
214	transmitters tending to be lost from the sea stars above c.a. 15 °C. Thus, relatively high
215	water temperatures might facilitate the loss of transmitters. However, water temperature
216	was higher during the latter period of the rearing experiments because they extended from

winter to summer; thus, we could not separate the effect of high water temperature on theloss of tags from that of the longevity of tags after installation.

219

Tracking study in the field

220Our results first show the two-dimensional movement of Northern Pacific sea stars in spring and summer. The hourly moving speed of the two sea stars in the summer of 2212222013 and the 10 sea stars in the spring of 2014 exceeded the HPE (6.4 m in 2013, 6.3 m 223in 2014). Overall, 91% and 84% of hourly moving speed in 2013 and 2014, respectively, 224were within the HPE. The HPE is usually overlooked in many study-target species that 225migrate distances that are longer than the HPE (e.g., many fish species, (Smith 2013)). 226However, the HPE must be considered for most benthic species because of their low 227 mobility. It very difficult to record behavioral movement accurately within the HPE, 228because the measurement and variance of the HPE are affected by marine conditions (e.g., tidal current and depth). Consequently, reducing the HPE represents a technological 229230challenge for future VPS studies of marine benthos.

The fastest daily moving speed of Northern Pacific sea stars was over 80 m/day in spring. Thus, sea stars can invade the scallop culture beds from outside areas located up to 1 km away from the bed within about two weeks. In Japan, newly seeded scallops are released on the scallop culture beds in spring, with all sea stars inhabiting the bed being removed with the dredge net beforehand. However, sea stars are only exterminated in a limited area within scallop culture bed. Our results show that cleaning of sea stars inside the culture fields alone is not sufficient, and that the extermination area should be expanded to surrounding areas to prevent rapid invasions and reduce the predation damage.

240The moving distance of Northern Pacific sea stars was significantly further in spring (2014) than in summer (2013). Furthermore, most individuals in spring and summer 241242moved in a specific direction. Gallagher et al. (2008) found that Asterias rubens migrated 243into their spawning area in spring, when seawater temperatures rose. For asteroid species, 244increasing seawater temperature is an important factor stimulating spawning activity (Hamel and Mercier 1995). In our study area, the spawning season of A. amurensis occurs 245246in May–July (Hada et al. 2003). Thus, the extended and directional movement distances of sea stars during spring might be related to spawning. 247248From summer to fall, the moving distance of Northern Pacific sea stars primarily 249remained within about 10 m/day. This moving distance was shorter than that documented

- during spring (>15 m/day). However, this difference might not be attributed to sea stars
 being less active in summer and fall compared to spring. Barbeau and Scheibling (1994)
 found that *A. rubens* consumed more numbers of small *P. maximus* scallops at 15 °C than
 - 16

253	at 4 °C or 8 °C. Furthermore, the predation rate of Asterias species increases with
254	increasing prey density (Barbeau et al. 1998). In our study, seawater temperature ranged
255	from 5.8 to 10.8 °C in spring, and from 14.3 to 18.1 °C in summer. The densities of
256	Japanese scallops estimated by dredge netting were 0.4 inds./m ² in spring and 0.8 inds./m ²
257	in summer. Thus, feeding conditions might be more suitable for Northern Pacific sea stars
258	in summer than in spring, during which time sea stars focus more on feeding than on
259	migration.
260	In conclusion, our study is the first to report the detailed movement of sea stars using
261	acoustic telemetry. We developed a reliable technique to monitor movement for up to two
262	months in the field, and showed variation in migratory and foraging activity over time.
263	Based on our findings, we suggest that it is not sufficient to only exterminate sea stars in
264	the immediate area where scallops are cultivated, due to their ability to migrate a long
265	distance in a short period of time; thus, to reduce predation rates and enhance productivity,
266	sea stars should be exterminated from larger areas.
267	
268	

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269

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Figure Captions

Figure 1. Location of the Abashiri Fisheries Science Center (a) and of the field study (b).

(b) contains a schematic of the receiver settings. Distance between receivers at Stn. 1–5 was set at 500 m in 2013 and 450 m in 2014 in the tracking study area.

Figure 2. Retention rate of transmitter for the sea stars in the rearing experiment.

Figure 3. Trace patterns of sea stars and the location of receivers (Stn. 1–5) in 2013 and 2014. Each symbol indicates the trace trajectory of each individual during the programmed period.







Fig. 2





Table

Experiment	Year	with / without tag	timing	n	Ray length	Growth rate	Wet weight	Growth rate
_					(cm)	(%)	(g)	(%)
Rearing	2013	with	before	28	12.6 ± 2.0		700.7 ± 55.7	
		with	after	25	12.9 ± 3.8	2.3 ± 4.2	693.8 ± 56.2	2.0 ± 0.7
		without	before	19	11.7 ± 2.5		716.7 ± 17.3	
		without	after	19	12.1 ± 2.7	0.9 ± 2.1	738.8 ± 28.5	3.1 ± 1.2
Field study	2013	with	before	7	15.3 ± 3.7		530.2 ± 138.9	
	2014	with	before	11	12.9 ± 1.6		346.7 ± 141.7	

Table 1 Summary of experimental sea stars, ray length (cm), wet weight (g), growth rate (%).

Organisms Year		n	Moving distance (m)	Moving speed (m day ⁻¹)			
Sea stars	2013	7	25.1 ± 18.9	4.3 ± 9.1			
	2014	11	90.9 ± 49.9	18.1 ± 15.2			
Dummy	2013	1	13.6	0.7			
	2014	1	8.9	0.6			

Table 2 Mean moving distance and moving speed during the programed period in the field study.

year	species	ID number	Range of	Mann-I	Kendall	R-value of Sp	earman's rank	Directi	ion	(°) of
		of each inds	s. speed (m h ⁻¹) te	st	correlation coefficient		movement		
				x axis	y axis	x axis	y axis	mean	±	SD
2013	Dummies	1	0.1 - 6.1					88.2	±	121.5
	Sea stars	2	0.1 - 8.0	+	+			98.5	±	72.5
		3	0.2 - 6.2	-	+			66.8	±	39.8
		4	0.1 - 8.7	+	-			79.3	±	74.6
		5	0.1 - 5.4		-			58.2	±	88.4
		6	0.1 - 16.3	-	-			91.2	±	29.9*
		7	0.1 - 5.7	+	+			38.6	±	81.3
		8	0.1 - 6.1	+	-			97.5	±	66.4
2014	Dummies	9	0.1 - 4.5					33.2	±	49.6
	Sea stars	10	0.1 - 17.4	+	+			65.4	±	18.1*
		11	0.1 - 45.9	-	+			48.4	±	42.5*
		12	0.1 - 7.7		-			45.4	±	22.6*
		13	0.1 - 8.3	+	-			15.9	±	39.2
		14	0.1 - 9.2	+	+			135.8	±	91.5
		15	0.1 - 18.4	-	+			58.6	±	10.5*
		16	0.1 - 7.4	+	+			323.5	±	33.2*
		17	0.1 - 7.9	-	-			317.5	±	42.6*
		18	0.1 - 6.7	+	+			261.6	±	13.5*
		19	0.2 - 7.1	+	+			22.4	±	42.1
		20	0.1 - 5.5	-	-			15.4	±	66.2

Table 3 Range of speed and results of each statistical analyses each specimen.

The asterisk indicates significant differences by each analysis (P < 0.05) during programed period."+" and "-" indicate that there were positive and negative trend in movement direction by Mann-Kendall test.

Blank cells indicate there was no significant trend statistically.