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

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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 ± 49.9 m) than in summer (25.1 ± 18.9 m), and that the moving speed was significantly faster in spring (18.1 ± 15.2 m/day) than in summer (4.3 ± 9.1 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**

2 Sea stars *Asterias* sp. intensively prey on bivalves, negatively impacting the
3 aquaculture of commercially important bivalves globally (Byrne et al. 2013). Therefore,
4 it is important to understand the motility of sea stars to quantify the potential predation
5 impact of sea stars on cultured bivalves, and, hence, the efficiency of various practices,
6 such as extermination. In Japan, North America, and Australia, sea stars are regularly
7 exterminated in grounds where bivalves are cultured. Freeman et al. (2001) and Gallagher
8 et al. (2008) indirectly estimated the migration distance of sea stars based on seasonal
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
12 sophisticated biotelemetry methods to monitor the location, behavior, and physiology of
13 free-ranging marine animals (Cooke et al. 2004). Biotelemetry provides a useful method
14 to monitor and collect data on the biology of animals that are not easily detectable, while
15 minimally influencing an animal's behavior. This approach allows the collection of more
16 data than is possible by other techniques, such as mark and recapture. It also allows
17 physiological and behavioral data collected in the laboratory and the field to be compared.
18 A method has already been developed to attach underwater acoustic transmitters to

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

The mariculture of Japanese scallops *Mizuhopecten yessoensis* has prospered on the eastern coast of Hokkaido Island, Japan, and has strong similarities with agriculture. The fishing 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 three to four years, the mature scallops are harvested by a dredge net, after which more young scallops are released in the emptied section again. Scallop mariculture is a very important fishery in east Hokkaido. However, the scallops are exposed to predation by sea stars, which has led to major economic issues (Imai 1978; Chiba and Arai 2014). 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 sea star *Asterias amurensis* individuals to nearly zero in these areas. However, new sea stars constantly invade the mariculture zone from outside areas to predate on scallops.

Therefore, it is important to gather direct information on the migration speed and seasonal behavioral patterns of Northern Pacific sea stars to implement effective density control measures. This study aimed to (1) develop a method of installing acoustic

transmitters on Northern Pacific sea stars, and (2) investigate the motility and behavior of sea stars in the scallop mariculture field using acoustic telemetry. Our results are expected to inform managers on the extent to which extermination practices should be conducted beyond the immediate culture area.

Materials and methods

Rearing experiment

In April and July of 2013, we conducted a rearing experiment to design an adequate method of installing acoustic transmitters on Northern Pacific sea stars. The sea stars ($n = 47$) were collected by towing a dredge net on the sea bottom (c.a. 35–50 m depth areas) of the Sea of Okhotsk off Abashiri Bay (Fig. 1a). Collected sea stars were transferred to a compact water tank ($L \times W \times H = 1.8 \times 0.9 \times 0.7$ m) set in the Abashiri City Fisheries Science Center. Natural sea water was pumped into each tank at about 0.7 l/h. Water temperature ranged from 1.8 to 17.8 °C.

Acoustic transmitters (model V9-1H, VEMCO Ltd., Halifax, Nova Scotia, Canada; 3.6 g in air, 9 mm in diameter, and 24 mm in length) were attached externally to the body surface of sea stars ($n = 28$, Table 1). Transmitters were fixed on the upper surface of the central part of a given arm with nylon fishing line (diameter = 0.37 mm) using the same

procedure developed for the knobby sea star (Chim and Tan 2013). The nylon fishing line was fixed on the arm from the central part of the upper surface to the inside ambulacral groove with small stainless-steel needles (diameter = 0.97 mm). Subsequently, the end of the line was fixed in the opposite direction. Thereafter, the transmitter was tied to the upper surface using the ends of the line. The knot of the line was sealed using two-component epoxy resin. This surgery was carried out twice per specimen; consequently, both ends of the transmitter were fixed to the surface of each sea star using nylon lines. A total of 14–22 juvenile scallops (shell length 4.9 ± 0.6 cm, weight 18.2 ± 5.4 g) were placed in each tank as prey for sea stars. When dead scallops were found, new scallops were added to maintain the initial density of scallops for about once a week during the rearing experiments.

Tracking study in the field

Study area

We conducted the field study in Nemuro Bay, which is located on the east side of Hokkaido (Fig 1). Most of Nemuro Bay is shallower than 20 m, with a profitable scallop-mariculture field covering a wide area of this bay. We set square study sites of 500×500 m in 2013, and 450×450 m in 2014 in an area of c.a. 2 km from the shoreline (depth

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

Acoustic telemetry and experimental animals

The position and movement of sea stars underwater were estimated using acoustic telemetry. For the VEMCO system, the method called VEMCO Positioning System (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-content/uploads/2013/09/understanding-hpe-vps.pdf>/ “Accessed 1 April 2017”). Before starting the animal tracking experiment, we estimated the horizontal position errors (HPE) when estimating the location of transmitters using a method that is exclusive to the 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.

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

transmitters (Fig. 1b). Acoustic transmitters (model V9-1H, 69 kHz, VEMCO Ltd., average pulse rate of 80 sec, pulse range 45–135 sec, expected battery life of 80 days) were immediately installed on seven and 11 sea stars in 2013 and 2014, respectively.

A dummy individual with no active movement was released in the same area to compare the rate of transmissions with live (moving) sea stars and to confirm that sea star movement was not just drift caused by sea currents. A dummy scallop (with the innards removed and sinkers added) also had a transmitter installed on the outside of the shell each year, respectively (shell length: 11.8 cm, wet weight 249.1 g in 2013, shell length: 12.6 cm, wet weight 236.0 g in 2014).

All specimens were reared in a water tank for c.a. 20 h. Before releasing the specimens, the diver removed all benthos (e.g., sea stars) from the central area of the study sites (around Stn. 5) and released the specimens every 10 m. Thereafter, divers released each sea star and scallop with attached transmitters in the center of the circles.

Statistical analyses

All data processing and statistical analyses were performed using the *R* statistical computing package (hereafter “*R*”; Version 3.2.0 Development Core Team, 2015). For 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 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 received by fewer than three receivers, data positions are not collected, and the interval of detecting the location of each specimen exceeds 30 min. After starting our experiments, the frequency of tracking location intervals increased from >30 min intervals to about two-week intervals. This study was designed to examine detailed and individual-based movement 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 frequency 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 (programmed period).

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

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

(1) Detection of active movement

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

(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

trends the movement from each successive tracked location was analyzed.

(3) Movement speed

The movement speed was estimated from moving distance of each specimen per unit time (hourly or daily) during the programmed period. The moving speed was compared between 2013 and 2014 with a Mann–Whitney U test. The 95% confidence interval of the mean movement speed was calculated by expanding the obtained data set using the boot strap method (Basic method), which was repeated 1000 times.

Results

Rearing experiment

Acoustic transmitters were retained for 71 days on the Northern Pacific sea stars. The retention rate of the transmitters on each day decreased by 20% for 73 days and by 50% for 90 days (Fig. 2). There was no significant difference in the growth rates (based on ray length and wet weight) of tagged and untagged starfish after the experiment ($p > 0.05$; Table 1).

Tracking study in the field

In 2013 and 2014, a total of 789,434 and 29,773 locations were detected for sea stars, respectively. The regularity of the tracking rate of the dummies fell below 90% on July 31 in 2013 and May 31 in 2014. Further migration analyses were conducted from July 23 to July 30 in 2013, and from May 23 to May 30 in 2014 (8 days; programmed period). A total of 6,226 and 10,645 sea star locations were recorded during the programmed period in 2013 and 2014, respectively.

Mean water temperatures ($\pm$ SD) in the experimental and programmed periods in 2013 were 16.3 ± 1.7 °C (range: 12.3–18.9 °C) and 14.2 ± 0.3 °C (range: 13.3–14.8 °C), respectively. In 2014, these temperatures were 8.5 ± 2.0 °C (range: 4.7–12.0 °C) and 5.8 ± 0.5 °C (range: 4.8–6.8 °C), respectively. Mean water velocities on the sea bottom in the experimental and programmed periods in 2013 were 8.8 ± 4.6 cm/s (range: 0.6–28.9 cm/s) and 17.1 ± 3.5 cm/s (range: 5.2–26.9 cm/s), respectively. In 2014, these velocities were 4.0 ± 3.4 cm/s (range: 0.8–20.7) and 8.3 ± 3.5 cm/s (range: 3.4–20.7 cm/s), respectively.

During the programmed periods, the mean ($\pm$ SE) total moving distance of Northern Pacific sea stars was 25.1 ± 18.9 m in 2013 and 90.9 ± 49.9 m in 2014 (Fig. 3, Table 2). There was a temporal trend in movement patterns on either the x or y axis for all sea stars in 2013 and 2014 (Table 3).

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

$p > 0.05$; Table 3); however, one other individual (#6) significantly moved in a northeasterly direction ($p < 0.05$). In 2014, seven sea stars showed significant directional movement ($p < 0.05$). Four of those individuals (# 10, 11, 12, and 15) tended to move in a northeastern direction and other three individuals (# 16, 17, and 18) tended to move in a northwestern direction. The direction of the dummies was random in each year.

Mean ($\pm$ SE) hourly moving speeds of Northern Pacific sea stars in 2013 and 2014 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 2013 and from 0.1 to 45.9 m/h in 2014 ($p > 0.05$; Table 3). The mean ($\pm$ SE) daily moving speeds of Northern Pacific sea stars in 2013 and 2014 were 4.3 ± 9.1 and 18.1 ± 15.2 m/day, respectively. In 2014, the daily moving speed was significantly faster than in 2013 ($p < 0.01$). The 95% bootstrap confidence intervals for the moving speed of sea stars in 2013 and 2014 were 3.2–14.6 and 17.2–29.9 m/day, respectively.

Discussion

Rearing experiment

In general, sea stars are sensitive to the external stimulation of their internal body parts, and have an extraordinary capacity to remove substances that intrude into their bodies (Olsen et al. 2015). In our study, acoustic transmitters were retained for 71 days.

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 °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 the loss of tags from that of the longevity of tags after installation.

Tracking study in the field

Our 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 2013 and the 10 sea stars in the spring of 2014 exceeded the HPE (6.4 m in 2013, 6.3 m in 2014). Overall, 91% and 84% of hourly moving speed in 2013 and 2014, respectively, were within the HPE. The HPE is usually overlooked in many study-target species that migrate distances that are longer than the HPE (e.g., many fish species, (Smith 2013)). However, the HPE must be considered for most benthic species because of their low mobility. It very difficult to record behavioral movement accurately within the HPE, because 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 challenge 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.

The moving distance of Northern Pacific sea stars was significantly further in spring (2014) than in summer (2013). Furthermore, most individuals in spring and summer moved in a specific direction. Gallagher et al. (2008) found that *Asterias rubens* migrated into their spawning area in spring, when seawater temperatures rose. For asteroid species, increasing seawater temperature is an important factor stimulating spawning activity (Hamel and Mercier 1995). In our study area, the spawning season of *A. amurensis* occurs in May–July (Hada et al. 2003). Thus, the extended and directional movement distances of sea stars during spring might be related to spawning.

From summer to fall, the moving distance of Northern Pacific sea stars primarily remained 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

at 4 °C or 8 °C. Furthermore, the predation rate of *Asterias* species increases with increasing prey density (Barbeau et al. 1998). In our study, seawater temperature ranged from 5.8 to 10.8 °C in spring, and from 14.3 to 18.1 °C in summer. The densities of Japanese scallops estimated by dredge netting were 0.4 inds./m² in spring and 0.8 inds./m² in summer. Thus, feeding conditions might be more suitable for Northern Pacific sea stars in summer than in spring, during which time sea stars focus more on feeding than on migration.

In conclusion, our study is the first to report the detailed movement of sea stars using acoustic telemetry. We developed a reliable technique to monitor movement for up to two months in the field, and showed variation in migratory and foraging activity over time. Based on our findings, we suggest that it is not sufficient to only exterminate sea stars in the immediate area where scallops are cultivated, due to their ability to migrate a long distance in a short period of time; thus, to reduce predation rates and enhance productivity, sea stars should be exterminated from larger areas.

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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. 1

Fig. 2

Fig. 3

Table

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

Experiment	Year	with / without tag	timing	n	Ray length (cm)	Growth rate (%)	Wet weight (g)	Growth rate (%)
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 2 Mean moving distance and moving speed during the programmed period in the field study.

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

year	species	ID number	Range of of each inds. speed (m h ⁻¹)	Mann-Kendall test		R-value of Spearman's rank correlation coefficient		Direction (°) of 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

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.