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Author(s)	Kanamori, Makoto; Baba, Katsuhisa; Natsuike, Masafumi; Goshima, Seiji
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6	Makoto Kanamori ^{a1, a2, c1} , Katsuhisa Baba ^{a3} , Masafumi Natsuike ^{a4} and Seiji Goshima ^{a2}
7	
8	^{a1} Hakodate Fisheries Research Institute, Fisheries Research Department, Hokkaido Research
9	Organization, 20-5, Benten, Hakodate, Hokkaido 040-0051, Japan
10	^{a2} Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido,
11	041-8611, Japan
12	^{a3} Fisheries Research Department, Hokkaido Research Organization, 38, Hamamachi, Yoichi,
13	Hokkaido 046-8555, Japan
14	^{a4} Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama,
15	Meguro, Tokyo 152-8550, Japan
16	^{c1} Correspondence should be addressed to: Makoto Kanamori, Hakodate Fisheries Research
17	Institute, Fisheries Research Department, Hokkaido Research Organization, 20-5, Benten,
18	Hakodate, Hokkaido 040-0051, Japan
19	email: kanamori-makoto@hro.or.jp
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21 ABSTRACT

22 The European sea squirt, Ascidiella aspersa was first found as an alien species in 2008 from Funka 23 Bay, Hokkaido, northern Japan, causing serious damage to the scallop aquaculture industry. We 24 investigated A. aspersa on cultured scallops and larval occurrence from July 2010 to June 2014 to clarify life history traits and population dynamics, and consider the relation between the life history 25 26 of A. aspersa and the process of scallop aquaculture. Larvae of A. aspersa were found from June to 27 December, and recruitment on cultured scallops occurred mainly between July and October. The 28 ascidians grew well and their weights increased until February. We found that 60-80% of A. aspersa 29 that had settled in summer had eggs or sperm in autumn, and 90–100% of A. aspersa matured early 30 the following summer. Maturity size in September was 17–20 mm as male, 22–24 mm as female. 31 Scallops in Funka Bay are hung in the spring and harvested from winter to the next spring. 32 Ascidiella aspersa settle as larvae in early summer, and grow well until winter, resulting in 33 overgrowth on scallops in the harvest season. The linking of the process of scallop aquaculture and 34 the life history of A. aspersa explains why this invasive ascidian has caused serious damage to the 35 aquaculture industry in the bay. In comparison to the earlier descriptions of the native population, A. 36 aspersa in Funka Bay has longer reproductive and growth periods, earlier initiation of reproduction, 37 and possibly smaller maturity size. 38 Keywords: invasive ascidian, Ascidiella aspersa, life history traits, population dynamics, aquaculture,

39 scallop

40 INTRODUCTION

41 Invasive ascidians have recently become a worldwide issue in coastal waters (Whitlatch & Bullard, 42 2007; Locke & Carman, 2009). More than 60 non-indigenous ascidians have been recorded in 43 tropical and temperate environments (Shenkar & Swalla, 2011). Non-indigenous ascidians have a rapid growth rate, short life span, and produce large numbers of short-lived non-feeding planktonic 44 45 larvae. These characteristics, combined with the lack of significant predators, allow ascidians to be 46 successful invaders (Shenkar & Loya, 2009). Ascidians can be strong spatial competitors and, once they become established, often experience population explosions that can develop into dense stands 47 48 or mats that overgrow and cover available surfaces (Whitlatch & Bullard, 2007). A recent increase in 49 shellfish aquaculture facilities has provided new surfaces (ropes, nets, cages, and shellfishes) for 50 colonisation by invasive ascidians, resulting in overgrowth and smothering of the shellfish (Lambert, 51 2007). For instance, heavy fouling by cryptogenic species, *Ciona intestinalis* (Linnaeus, 1767), was 52 associated with higher mussel mortality and lower overall size in Nova Scotia (Daigle & Herbinger, 53 2009). In addition, even if the ascidians have no negative effects on the bivalves directly, removal of 54 the invasive species is costly and requires additional labour by aquaculturists (Carman et al., 2010). 55 The mussel aquaculture industry has been overwhelmed by extremely large numbers of the invasive 56 ascidian Styela clava Herdman, 1881 in Prince Edward Island (Bourque et al., 2007), resulting in 57 increased production costs estimated at \$4.5 million per annum (Shenkar & Swalla, 2011). In Japan, 58 some non-indigenous ascidians have been reported, such as Molgula manhattensis (DeKay, 1843) 59 and Polyandrocarpa zorritensis (Van Name, 1931) (Tokioka & Kado, 1972; Nishikawa et al., 1993).

60	However, no significant effects of invasive ascidians had been noted on the ecosystems or fisheries
61	prior to the appearance of Ascidiella aspersa (Müller, 1776) (The Plankton Society of Japan and The
62	Japanese Association of Benthology, 2009; Kanamori et al., 2012; Nishikawa et al., 2014).
63	The European sea squirt, A. aspersa, is a solitary marine and estuarine ascidian that is native from
64	Norway to the Mediterranean (Berrill, 1950; de Kluijver & Ingalsuo, 2004; Mackenzie, 2011). The
65	species has been introduced to North and South America, India, Australia, New Zealand, South
66	Africa, South Korea, and Japan (Brewin, 1946; Kott, 1985; Nagabhushanam & Krishnamoorthy,
67	1992; Carlton, 2000; Robinson et al., 2004; Tatián et al., 2010; Kanamori et al., 2012; Pyo et al.,
68	2012; Nishikawa et al., 2014). Because there are no efficient predators, A. aspersa can form large
69	populations and subsequent high amounts of biomass, which redirects energy to decomposers and
70	not to higher trophic communities (Currie et al., 1998). In addition, colonisation by A. aspersa
71	reduces available substrata on which other species recruit successfully (Osman & Whitlatch, 2000).
72	These characteristics have the potential to significantly affect species composition, reducing overall
73	biodiversity (Mackenzie, 2011). Ascidiella aspersa also competes directly with other native filter-
74	feeders, including economically important species such as scallops, mussels, and oysters (Currie et
75	al., 1998). Therefore, A. aspersa is listed in the Global Invasive Species Database (2010), which is
76	managed by the International Union for Conservation of Nature and Natural Resources, to increase
77	awareness and to facilitate effective prevention and management activities.
78	The Japanese scallop, Mizuhopecten yessoensis (Jay, 1856), is one of the most important seafood
79	species in Japan (Kosaka & Ito, 2006; MAFF, 2015). Funka Bay, located in southwestern Hokkaido,

80	is one of the main commercially productive areas for scallop culture in Japan, where predominantly
81	suspension culture techniques are used (Kosaka & Ito, 2006). The method for culturing is called
82	'Mimi-zuri' or ear-suspended method: a small hole is drilled at the front-eared beak of the left valve
83	and the scallop is hung on a rope by using artificial strings or plastic clips (Kosaka & Ito, 2006).
84	In September 2008, A. aspersa was first found densely covering cultured scallops in Funka Bay,
85	severely damaging aquaculture activities by causing the facility to sink and the scallops to fall off,
86	and increasing expenses due to the need to dispose of the invasive species (Kanamori et al., 2012;
87	Nishikawa et al., 2014). The ascidians overgrowing cultured scallops in Funka Bay had been
88	correctly identified as A. aspersa through observation of the characteristics of internal morphology,
89	follicle cells of egg, and DNA analysis of mitochondrial cytochrome c oxidase subunit I (Kanamori
90	et al., 2012; Nishikawa et al., 2014). This is regarded as the first record of A. aspersa in the northern
91	Pacific Ocean (Nishikawa et al., 2014). In South Korea, Pyo et al. (2012) identified many specimens
92	collected in 2010 and 2011 as A. aspersa by using morphological and molecular analysis, and
93	concluded that A. aspersa was widespread along three coastlines of Korea. However, the relationship
94	between the Japanese and the Korean populations is unknown. In Japan, A. aspersa has been found
95	in Hokkaido, Aomori, Iwate, and Miyagi Prefectures, and has become one of the most serious
96	problems for bivalve aquaculture in northern Japan (Figure 1, Kanamori et al., 2014). Basic
97	information such as reproductive season, growth patterns, maturity size, and population dynamics of
98	A. aspersa in Japanese invasive populations is critical to controlling their impact.
99	In this study, we examined the recruitment, growth, maturity, and population dynamics of A .

100	aspersa on cultured scallops in Funka Bay, and sought to relate the life history of A. aspersa with
101	scallop aquaculture, to understand why the invasive ascidian has become a serious problem for the
102	aquaculture industry in the bay. We also compared our results with a past study of native populations
103	by Millar (1952), which is considered the most detailed account of the reproductive cycles of A .
104	aspersa (Global Invasive Species Database, 2010), to deepen our understanding of the life history
105	traits of this global invasive ascidian.
106	
107	MATERIALS AND METHODS
108	Larval density and seawater analyses
109	In preparation for our study, we observed the morphology of larvae and their changes during
110	metamorphosis in the laboratory. Monthly larval surveys were conducted from July 2010 to June
111	2014 at the sampling station ($42^{\circ}16.208'$ N, $140^{\circ}20.568'$ E, Depth = 32 m, Figure 2) to determine the
112	reproductive period of A. aspersa. Larvae were collected in 225-mm or 300-mm diameter plankton
113	nets (NXX13 nylon mesh, opening of 100 μ m, RIGO CO. LTD) hauled vertically from the bottom
114	by hand. Our surveys were conducted between 11:30 and 13:30. Samples were fixed with
115	glutaraldehyde (final concentration: 1%), and observed by stereoscopic microscope to count the
116	number of A. aspersa larvae.
117	To determine the environmental factors that affect A. aspersa populations, water temperature and
118	salinity were measured at every 1 m by CTD (RINKO-Profiler ASTD102, JFE Advantech Co. Ltd),
119	and 300 mL of seawater was sampled using a Van Dorn sampler (RIGO CO. LTD) at depths of 5, 10

120	and 15 m at the sampling station. Each sample was filtered using a glass microfiber filter (GF/F, 47
121	mm, Whatman, GE Healthcare Life Science), and chlorophyll a (Chl-a) was extracted with 10 mL of
122	N,N-dimethylformamide (DMF) (Wako Pure Chemical Industries, Ltd.). The Chl-a content was
123	measured from the change using fluorescence (excitation 436 nm, emission 660 nm) before and after
124	acidification by adding 0.1 mL of 5% HCl in 3 mL of the sample DMF solution. Fluorescence was
125	measured by a fluorescence spectrophotometer (FP6300, JASCO Corp.). The concentration of Chl-a
126	was calculated using Chl-a from chlorella (Wako Pure Chemical Industries, Ltd.) as the standard.
127	
128	Sampling, measurement, and maturation level of A. aspersa
129	Five scallops, Mizuhopecten yessoensis, were collected monthly at 5-, 10-, and 15-m depths from a
130	culture rope near the sampling station between July 2010 and June 2014 (total 15 scallops were
131	collected monthly). In Funka Bay, scallops are produced from a natural population of larvae, from
132	spring to summer. Scallops are reared in cages from autumn to spring, and this is called the
133	intermediate culture. Juvenile scallops, after an intermediate culture, are suspended for hanging
134	culture in spring. Collection of the scallops is initiated after spring (June or July) each year, and
135	completed the following June (from July 2010 to June 2011, from June 2011 to June 2012, from
136	June 2012 to June 2013, and from June 2013 to June 2014). When hanging cultures are started, A.
137	aspersa are seldom found on the scallops, which means that the ascidians found on scallops after
138	spring are newly settled. In this study, therefore, the life history traits and population dynamics of A.
139	<i>aspersa</i> were surveyed through four generations, the 2010, 2011, 2012, and 2013 cohorts. 7

140	Each scallop was placed in a zippered plastic bag to prevent the ascidians from falling off and
141	carried to the laboratory in a cooler box. The surface of scallop was examined by direct observation
142	and under a stereoscopic microscope. Ascidiella aspersa were removed using forceps. The number
143	of individuals per each scallop was counted to assess seasonal variation in abundance, and the wet
144	weight of A. aspersa was measured to assess seasonal variation in biomass. The weight of each
145	scallop was quantified to compare it with the weight of the ascidians attached to it. Body length of
146	each ascidian was determined within 0.1 mm using digital vernier calipers to examine size structure
147	and growth. For small individuals (body length < 5 mm) found using a microscope, body length was
148	measured from images captured using a Digital Sight Ds-Fi1 camera with NIS-Elements software
149	(Nikon Corporation). More than 50 A. aspersa were randomly chosen from all depths in September,
150	December, March, and June in 2010, 2011, and 2012, and fixed in 5-10% formalin seawater. After
151	measuring body length, the specimen was dissected and genital ducts examined for eggs and sperm
152	to evaluate the maturity. The 2013 cohort was not examined in terms of maturity. Sizes during
153	maturity as male and female in September were analysed using generalised linear model (GLM)
154	with a binomial error distribution. The response variable was whether eggs or sperm were in the
155	ducts; the explanatory variable was body length, by using the statistical software R version 3.01 (R
156	Development Core Team, 2013).
157	

160 RESULTS

161 Larval density and environmental factors

162 The larvae of *A. aspersa* appeared in July–December 2010, July–November 2011, June-December

163 2012, and June–December 2013 (Figure 3), and were not found in the samples from January to May

164 each year. Densities (individuals/m³) were the highest between July and October. The highest density

165 in each year was 74.3 in October 2010, 95.5 in August 2011, 37.7 in September 2012, and 22.6 in

166 July 2013. Data were not collected in December 2012 because the plankton net was broken during

167 the survey.

168 Water temperature reached its peak in August or September, except at 15-m depth in 2010 (Figure 169 4A). During summer 2010, a strong thermocline developed, in which the water temperature in 170 August at 5-m depth was 23.9°C, whereas at 15-m depth, it was only 12.9°C. After the thermocline 171 dissipated, the maximum water temperature at 15-m depth was 17.5°C, recorded in October. Water 172 temperature was the lowest in February or March at all depths, in the range of 2.0–3.2°C. The 173 seasonal fluctuation in salinity was stable in comparison with that of water temperature (Figure 4B). 174 From spring to summer, the salinity was relatively low, fluctuating from 31.0 to 33.0 in part because 175 of the inflow of the Oyashio Current, with low salinity, and in part because of the discharge of land 176 water, including snowmelt runoff (Ohtani et al., 1971a). From autumn to winter, the salinity 177 fluctuated from 33.0 to 34.0 because of the inflow of the Tsugaru Warm Current, with high salinity 178 (Ohtani et al., 1971b). There were no obvious differences in salinity between depths, except in 179 August-September 2010, when the thermocline developed intensely. A strong increase in Chl-a, a

180 spring bloom, occurred between February and April every year, and the concentration of Chl-a

181 peaked at 6–8 µg/L (Figure 4C). After the spring bloom, the concentration remained low in summer

182 and had an annual variability in autumn. A difference in Chl-a concentration between depths was not

183 noted, and the average concentrations in 5-, 10-, and 15-m depths through the survey period were

184 nearly the same at 1.53, 1.49, and 1.51 μ g/L, respectively.

185

186 Seasonal variation in size, weight, and maturity of *A. aspersa* on scallops

187 In June, few A. aspersa were found on cultured scallops, and the average number per scallop at all 188 depths was 0–0.9 individuals. In July, the average number increased to 0.9–7.8 individuals per 189 scallop, and A. aspersa was observed at all depths except at the 5-m depth in 2013. After July, the 190 number of A. aspersa per scallop increased and reached its peak between August and October. The 191 average number of A. aspersa per scallop at each depth in each year is shown in Figure 5. The 192 maximum number per scallop on average for all depths in each year was 117.4 individuals in 193 October 2010, 39.2 individuals in August 2011, 22.9 individuals in September 2012, and 45.7 194 individuals in August 2013. During the time the numbers were increasing, as water depth increased, 195 the number of A. aspersa also increased. After that, their numbers decreased, with an especially rapid 196 rate of decrease at the 15-m depth. Because of this trend, in winter, the difference in number between 197 the 10-m and 15-m depths became small. The number of A. aspersa at the 5-m depth was relatively 198 low throughout the survey. June 2011 abundance data are not represented because only five scallops 199 were collected, without the depth information.

200	No clear variation in size structure of <i>A. aspersa</i> on cultured scallops was noted between depths.
201	However, seasonal variation in the size frequency was noted when all depths were combined, as
202	shown in Figure 6. Juvenile ascidians (body length < 5 mm) dominated during the period of
203	increasing abundance. For the 2010 cohort, many juvenile ascidians were found from August to
204	October, whereas for the 2011, 2012, and 2013 cohorts, juvenile ascidians were found mainly from
205	July to August. Figure 7 shows the seasonal variation in the body length of A. aspersa on cultured
206	scallops at all depths. Ascidiella aspersa grew well until February following each season, when their
207	body length remained unchanged or decreased slightly from February to March or April.
208	The biomass of the scallops increased steadily in each year. The biomass of A. aspersa on scallops
209	increased, with fluctuations, until February, and after that, changes were less clear (Figures 8, 9). For
210	the 2010 cohort, the average weight of A. aspersa at all depths exceeded that of the scallops even in
211	November, and was three to seven times heavier in harvest season, from December to April,
212	meaning that the weight of A. aspersa accounted for 75–90% of the total weight of the harvest. For
213	the 2011 cohort, the average weight of the ascidians was less than that of the scallops except in
214	February and March. For the 2012 cohort, the average weight of the ascidians was always less than
215	that of the scallops. The weight of the ascidians in the 2013 cohort was more than that of the scallops
216	in and after November. June 2011 weight data are not represented because only five scallops were
217	collected, without the depth information.
218	Ascidiella aspersa with eggs and sperm in the ducts were found as late as September 2010, 2011,

and 2012 (Figure 10). Because there were many juvenile ascidians in September 2010, the ratio of

220	ascidians having gametes was low (15%) at that time. On the other hand, in 2011 and 2012, the ratios
221	were high, at 72.2% and 62.3%. Although there were few larvae and juveniles in December and
222	March, many ascidians had eggs or sperm in the ducts. The ratios of individuals having gametes in
223	December 2010, 2011, and 2012 were 52.0%, 81.6%, and 78.0%, respectively, and, in March 2011,
224	2012, and 2013, the ratios were 54.6%, 84.4%, and 87.5%, respectively. In June 2011, 2012, and
225	2013, the ratios were 92.1%, 100%, and 100%, respectively. In September, estimated 50% maturity
226	size as male was 17–20 mm, and as female, 22–24 mm (Figure 11). The maturity size as female was
227	approximately 5 mm larger than that as male, and in December and March, there were many A.
228	aspersa with no gametes, even if the body length exceeded the 50% maturity size estimated in
229	September. In the GLM analysis of maturity related to size as male and female in September, all of
230	the estimated coefficients for body length were significant (Table 1, Wald test $P < 0.001$).
231	
232	DISCUSSION
233	Life history traits and population dynamics of A. aspersa in Funka Bay
234	In Funka Bay, the larvae of A. aspersa appeared between June and December, and the highest
235	density was observed between July and October. In addition, juvenile ascidians were found on
236	cultured scallops mainly between July and October. Therefore, the reproductive period of A. aspersa
237	is thought to be from June to December, and the main breeding season, from July to October. A study
238	conducted from 1991 to 1997 in Long Island Sound, New England, showed that recruitment of A.
239	aspersa started between June and July and, on average, initiation of recruitment was estimated to

240	occur on 1 July (Stachowicz et al., 2002). The onset of recruitment of A. aspersa in Funka Bay
241	corresponds to that in Long Island Sound. The reproductive season of ascidians usually coincides
242	with the period of maximum food production (Lambert, 2005). However, this idea does not apply to
243	A. aspersa in Funka Bay because it is between February and April that the bay has a spring bloom
244	and conditions for filter-feeders are good. Ascidiella aspersa grew well until February following the
245	reproductive season. Their body length remained stagnant from February to March or April, when
246	the bay has high production. The Oyashio Current, a subarctic current, introduces cold water to the
247	bay and water temperatures fall below 4°C in February and March. Hence, the growth of A. aspersa
248	would be depressed by low water temperature.

249 In autumn, A. aspersa had eggs or sperm. Ascidiella aspersa are known to be hermaphroditic, 250 although the male sex organs develop first (Millar, 1952). In Funka Bay, the maturity size as males 251 was estimated to be 17-20 mm, and as females was estimated to be 22-24 mm. Ascidians that 252 reached these sizes in autumn had gametes and were expected to start reproduction. In December 253 and March, there were many immature ascidians whose body length was greater than the maturity 254 size in September. This indicated that factors other than body length influenced the accumulation of 255 gametes. Because larvae and juvenile ascidians were scarcely found in winter and spring, the 256 ascidians having gametes in December and March are thought to be the animals that reach maturity 257 size in autumn and continue to have gametes after the reproductive season. In June, most of the A. 258 aspersa had eggs and sperm, showing that the conditions needed for the maturity are fulfilled 259 between March and June. Temperature is correlated with the timing of reproduction in many ascidian

260	species (Millar, 1971; Goodbody, 2004; Shenkar & Loya, 2008; Rius et al., 2009). The average
261	temperatures found at 5–15-m depth in September, December, March, and June were 21.3°C, 8.0°C,
262	2.6°C, and 11.2°C, respectively. Hence, A. aspersa stopped gamete accumulation when water
263	temperature decreased from 21.3°C to 8.0°C, and started it again when water temperature increased
264	from 2.6°C to 11.2°C. From this, we speculate that A. aspersa have a critical temperature to start or
265	stop the gamete accumulation, estimated to be between 8 and 11°C.
266	The number of A. aspersa on the cultured scallops increased sharply after July and the number of
267	juvenile ascidians increased with increasing water depth. In most cases, larval behaviour is a good
268	predictor of adult distribution of ascidians (Svane & Young, 1989). At first, the larvae of A. aspersa
269	exhibit positive phototaxis and negative geotaxis; however, the reactions are reversed at later stages
270	(Niermann-Kerkenberg & Hoffman, 1986). The reaction of larvae of A. aspersa to environmental
271	factors may explain the difference in quantity of ascidians at varying depths in our results. In our
272	survey, the number of juvenile ascidians did not increase in autumn 2011, 2012, and 2013, although
273	the generation from the previous year would continue reproduction; moreover, the recruits in
274	summer would start spawning in autumn. Ascidiella aspersa and other fouling animals settled over
275	the surface of scallops in summer, and they may have prevented larvae of A. aspersa from settling on
276	scallops in autumn.
277	In 2010, the increase in ascidians was the greatest from August to September; however, in other
278	years, it was from July to August. In Funka Bay, warm and less saline water is found in the surface

layer from spring to summer, and a strong seasonal thermocline is formed (Ohtani et al., 1971a). The 279

280	thermocline dissipates by atmospheric influences and inflow of the Tsugaru Warm Current from
281	summer to autumn (Ohtani et al., 1971b). The average air temperature of northern Japan in summer
282	2010 was the highest it had been since 1946 (Japan Meteorological Agency, 2010a), and in autumn,
283	the temperature continued to be higher than that in an average year (Japan Meteorological Agency,
284	2010b). In addition, the inflow of the Tsugaru Warm Current was delayed, and not observed until
285	mid-September (Hakodate Fisheries Research Institute, 2010). Under these conditions, the strong
286	thermocline developed for a long time, and water temperatures in the depths below 15 m did not
287	increase in summer. The low water temperature at deeper zones in summer 2010 may have
288	influenced the reproduction of A. aspersa populations, resulting in the delay of A. aspersa increasing
289	on cultured scallops.
290	During our survey, the Great East Japan Earthquake and the subsequent tsunami occurred on 11
291	March 2011. Funka Bay is approximately 500 km away from the centre of shock. Even so, the
292	waves (maximum 1.6-m high) repeatedly struck the bay, damaged the facilities for scallop
293	aquaculture, and affected coastal fauna (Japan Meteorological Agency, 2012; Natsuike et al., 2014).
294	Most of the A. aspersa on the scallops at 5-m depth disappeared in and after March 2011 because the
295	tsunami caused ascidians to drop off scallops in the shallow water. The effect on the ascidian
296	population at 10-15-m depth appears small. The facilities damaged by the tsunami were removed
297	and new facilities were established between 2011 and 2012 (Hokkaido Government, 2012), and
298	consequently, many ascidians attached to the facilities were also removed. Facilities of aquaculture
299	are considered important habitats for invasive ascidians (Lambert 2005; Howes <i>et al.</i> 2007; Carman 15

et al. 2010). The tsunami and the removal of damaged facilities may explain why the numbers of *A*. *aspersa* on the scallops decreased in 2011 and 2012.

302

303 Life history of A. aspersa and the process of scallop aquaculture

304 The surface of newly suspended scallops is clean because they rub against netting or other scallops in 305 the cage during intermediate culture; thus, they become a suitable substrate for sessile organisms, 306 especially species that begin reproduction in early summer, such as A. aspersa. Harvest season for 307 cultured scallops in the bay is mainly from December to April in order to avoid the shellfish toxin 308 period and competition with other areas of production (Imai et al., 2014). Consequently, there is 309 enough time for A. aspersa that have settled in summer to grow prior to scallop harvesting, and 310 hence the harvest and shipment must be conducted after the weight of ascidians become several 311 times heavier than that of scallops. The linking of "hang in spring and harvest in winter" of the 312 cultured scallops and "recruitment after spring and rapid growth until winter" of A. aspersa results in 313 serious problems in the aquaculture industry in Funka Bay (Figure 12). Effects of invasive organisms 314 on an aquaculture industry depend on the relationship between the life history of the invasive species 315 and the process of aquaculture in the introduced area. It is important to understand the life history 316 and adaptations of invasive species in order to evaluate the risk of introduction to fisheries activities. 317

318 Comparison of life history of A. aspersa in Funka Bay and native area

319 The article by Millar (1952) is considered to be the most detailed account of the life history of A.

aspersa (Global Invasive Species Database, 2010), and the description in the literature and many
databases are based on this significant work (e.g. Global Invasive Species Database, 2010;
Mackenzie, 2011). Millar (1952) studied the reproductive cycle and population dynamics of *A*. *aspersa* throughout 1950 and 1951 in Ardrossan, southwestern Scotland, which is their native habitat
and we summarise his findings here.
Larvae settle in the summer (July–August) and grow until the end of September. *Ascidiella*

326 aspersa grow again after winter or spring. The life span is on the order of 18 months, extending 327 approximately from the middle of one summer until the winter of the following year. Ascidiella 328 aspersa have only one spawning season, and that is in the year after A. aspersa settled as larvae. 329 Ascidiella aspersa is hermaphroditic and protandrous, in which the male reproductive organs come 330 to maturity before the female reproductive organs. Sexual maturity is dependent on size; sperm 331 development occurs when the animals are about 25-mm long, while eggs are found in the oviduct 332 when the animals are about 30-mm long (Millar 1952). Most of the life history traits of A. aspersa in 333 Funka Bay seem to be essentially identical to that summarised by Millar (1952). However, there are 334 some clear differences.

335 The estimated reproductive period (June–December) and the main breeding season (July–

October) in Funka Bay is longer than the recruitment season in Ardrossan (July–August). *Ascidiella aspersa* grow well until February in Funka Bay, and the average water temperature at 5–15-m depth fluctuates between 4 °C and 21°C from July to February. In Ardrossan, *A. aspersa* grow until late in September. From the information in Saltcoats, a town near Ardrossan, the peak water temperature is

340	14°C in August, and the lowest is 7 °C in March (World Sea Temperatures, 2015). This suggests that
341	factors other than water temperature influenced the differences in growth period of A. aspersa
342	between Funka Bay and Ardrossan. In Funka Bay, 60–70% of A. aspersa settled in summer have
343	eggs or sperm in September, and A. aspersa would start to reproduce. From January to May, A.
344	aspersa stop reproduction, and start spawning again in June. In contrast, A. aspersa in Ardrossan is
345	regarded as the typical annual species, which has only one spawning season in the year after it has
346	settled. Further, the extra generation of A. aspersa does not occur in the native population on the west
347	coast of Norway (Dybern, 1969). The natural distribution of A. aspersa includes European low
348	latitudes, such as the Mediterranean, but we have no information about the reproduction of A.
349	aspersa in these areas. Ascidiella aspersa populations in the warmer temperature of the native range
350	perhaps start to reproduce in the recruitment year as seen in Funka Bay. There is a possibility that the
351	voltinism and reproductive traits of A. aspersa population is directly influenced by the habitat
352	temperature, as discussed in the case of peracarida crustaceans (e.g. Vincente & Sorbe, 2013). Study
353	of the life history and population dynamics of native A. aspersa population in warmer habitats is
354	required to understand the life history strategy of this species.
355	The maturity size of A. aspersa in Funka Bay is approximately 5–8 mm smaller than that in
356	Ardrossan. In Millar's study, the samples were fixed after they were narcotised with menthol; in our
357	study, the samples were directly fixed, which may have led to an underestimation of the body length.
358	The test of A. aspersa is firm, and their siphons are short. Consequently, the difference in body length
359	between individuals narcotised and those not narcotised was small, up to 3.5 mm, when the body 18

360	length was from 10.3 to 44.6 mm (N = 30, examined by MK on 14 September 2015). The
361	differences in method of fixation would not fully account for the disagreement of maturity size
362	between Funka Bay and Ardrossan. Millar (1952) also described that ascidians in Loch Sween,
363	Argyll, western Scotland, became mature at a smaller body size than did those in any of the samples
364	from Ardrossan. Further analysis is required to determine whether maturity size is different between
365	Funka Bay and native ranges.
366	As described above, compared with the native population in Ardrossan, A. aspersa in Funka Bay
367	has a longer reproductive and growth period, earlier initiation of reproduction, and possibly smaller
368	maturity size. The vigour and success of invasive species has been explained by favourable
369	environments where they are introduced and by release from natural enemies and the adaptation or
370	evolution of increasing competitive ability (Blossey & Nötzold, 1995, Keane & Crawley, 2002;
371	Colautti et al., 2004). Further studies that assess environmental factors, such as temperature and food
372	conditions, and enemies regulating the population in native regions, are necessary to compare life
373	history traits of the global invasive species, A. aspersa, in native and introduced ranges.
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537 FIGURE LEGENDS

539	Fig. 1. Cultured scallops, Mizuhopecten yessoensis, overgrown by the invasive ascidian, Ascidiella
540	aspersa, in Funka Bay, Hokkaido, northern Japan: (A), (B) a cultured rope with scallops hung by
541	using plastic clips; (C) a cultured scallop held in the hand, having shell length of approximately 90
542	mm. More than 30 ascidians were attached to the scallop in (C) when the photos were taken on 18
543	May 2015.
544	
545	Fig. 2. Maps showing Funka Bay, Hokkaido, northern Japan and a sampling station (42°16.208'N,
546	$140^{\circ}20.568$ 'E, Depth = 32 m). Recording of environmental conditions and plankton surveys were
547	conducted at the sampling station. Cultured scallops were collected around the sampling station to
548	investigate the attached Ascidiella aspersa.
549	
550	Fig. 3. Seasonal variation in larval density of Ascidiella aspersa at a sampling station (42°16.208'N,
551	140°20.568′E, Depth = 32 m), Funka Bay, Hokkaido, northern Japan from July 2010 to June 2014. J,
552	S, N, J, M, M: July, September, November, January, March, May.
553	
554	Fig. 4. Seasonal variation in (A) water temperature, (B) salinity, and (C) chlorophyll a concentration
555	at a sampling station (42°16.208'N, 140°20.568'E, Depth = 32 m), Funka Bay, Hokkaido, northern
556	Japan from July 2010 to June 2014. J, S, N, J, M, M: July, September, November, January, March,

May.

559	Fig. 5. Seasonal variation in the number of Ascidiella aspersa on cultured scallops. (first J on the
560	horizontal axis is June of the year presented on the graph; last J is June of the following year).
561	Average and standard error of number of <i>A. aspersa</i> on a scallop in each depth are shown: (A) 2010
562	cohort from July 2010 to May 2011; (B) 2011 cohort from June 2011 to June 2012; (C) 2012 cohort
563	from June 2012 to June 2013; and (D) 2013 cohort from June 2013 to June 2014. For June 2012 and
564	2013, cultured scallops hung in the previous year and the year were collected. Scales of vertical axes
565	are different.
566	
567	Fig. 6. Seasonal variation in size frequency of Ascidiella aspersa on cultured scallops at all depths.
568	
569	Fig. 7. Seasonal variation in the body length of Ascidiella aspersa on cultured scallops at all depths
570	(first J on the horizontal axis is June of the year presented on the graph; last J is June of the following
571	year). The medians are shown as representative values. Bars indicate 25th and 75th percentiles: (A)
572	2010 cohort from July 2010 to June 2011; (B) 2011 cohort from July 2011 to June 2012; (C) 2012
573	cohort from June 2012 to June 2013; and (D) 2013 cohort from July 2013 to June 2014.
574	
575	Fig. 8. Seasonal variation in biomass of Ascidiella aspersa on cultured scallops (first J on the
576	horizontal axis is June of the year presented on the graph; last J is June of the following year).

Average and standard error of wet weight (w.w. in grams [g]) of *A. aspersa* per month at each depth
is shown: (A) 2010 cohort from July 2010 to May 2011; (B) 2011 cohort from June 2011 to June
2012; (C) 2012 cohort from June 2012 to June 2013; and (D) 2013 cohort from June 2013 to June
2014. For June 2012 and 2013, cultured scallops hung in the previous year and the year were
collected. Scales of vertical axes are different.

582

Fig. 9. Seasonal variation in biomass of *Ascidiella aspersa* and cultured scallop, *Mizuhopecten yessoensis* (first J on the horizontal axis is June of the year presented on the graph; last J is June of the following year). Average wet weight (w.w. in grams [g]) of *A. aspersa* and *M. yessoensis* per month at all depths is shown: (A) 2010 cohort from July 2010 to May 2011; (B) 2011 cohort from June 2011 to June 2012; (C) 2012 cohort from June 2012 to June 2013; and (D) 2013 cohort from June 2013 to June 2014. Scallops were hung in spring each year. For June 2012 and 2013, cultured scallops hung in the previous year and the year were collected. Scales of vertical axes are different.

Fig. 10. Size frequency and the presence of sperm and eggs in the ducts of *Ascidiella aspersa*: (A)
2010 cohort; (B) 2011 cohort; and (C) 2012 cohort. Ascidians having neither eggs nor sperm in their
ducts are regarded as immature.

594

Fig. 11. Relation between body length and maturity of *Ascidiella aspersa* in September. Maturity is
assessed by the presence of gametes in the ducts. The best-fit logistic curves are shown. Maturity

size (M₅₀) indicates the size at which 50% of *A. aspersa* mature, estimated according to the logistic
curves.

- 600 Fig. 12. Life history of Ascidiella aspersa and basic process of scallop culture in Funka Bay,
- 601 Hokkaido, northern Japan. Scallops hung in spring become suitable substrate for A. aspersa, which
- 602 start their reproduction in early summer. The rapid growth and weight gains of A. aspersa from
- 603 summer to winter cause serious problems for the scallop-harvesting season.













Fig.6









Body length (mm)



Fig.11

	Winter	Spring	Summer	Autumn	Winte	er Spring	Summer			
Life A. as	history spersa	of	Recruitme (Peaks; Ju	cruitment eaks; JulSep.)						
Growing (Rapidly; JulFeb.)										
				Reproduct	ion	Reproduction				
Proc scall	ess of op cult	ure	Many a the nev	scidians atta vly cultured s	ch to callops.	Weight gains of the ascidians cause the problem for harvesting.				
Inte	ermediate	e	Ļ			,				
cult (Oc	ure tFeb.)	Ear-hangin (MarMay	g Hang) (Ju	ing culture nNov.)	Ha (D	ecApr.)				

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Table 1. Results of generalized linear model (GLM) analysis for the maturity ofAscidiella aspersa collected in September. All of the coefficients for body length aresignificant (P < 0.001, Wald test). The maturity size indicates the size at which 50% of A.aspersa mature.

As male	Explanatory variable								
		Interce	ept (β_0)		Body length (β 1)				
	Coef.	SE	Z	р	Coef	SE	Z	р	(- β ₀ /β ₁)
2010	-13.612	3.563	-3.820	< 0.001	0.681	0.185	3.678	< 0.001	20.0
2011	-8.850	2.382	-3.715	< 0.001	0.474	0.121	3.901	< 0.001	18.7
2012	-5.471	1.087	-5.003	< 0.001	0.320	0.059	5.431	< 0.001	17.1
As female	Explanatory variable								
	Intercept (β_0)				Body length (β_1)				size (mm)
	Coef.	SE	Z	р	Coef	SE	Z	р	(- β ₀ /β ₁)
2010	-13.962	3.338	-4.183	< 0.001	0.583	0.145	4.016	< 0.001	23.9
2011	-10.238	2.359	-4.340	< 0.001	0.473	0.107	4.415	< 0.001	21.7
2012	-8.450	1.483	-5.697	< 0.001	0.351	0.065	5.4506	< 0.001	24.1