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1 Running Head: Life history traits of *Ascidrella aspersa* in Japan

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3 **Life history traits and population dynamics of invasive ascidian, *Ascidrella aspersa*, on cultured**  
4 **scallops in Funka Bay, Hokkaido, northern Japan**

5

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20

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*  
25 *clarify life history traits and population dynamics, and consider the relation between the life history*  
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  
44 rapid growth rate, short life span, and produce large numbers of short-lived non-feeding planktonic  
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  
47 they become established, often experience population explosions that can develop into dense stands  
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 *Ascidella 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). *Ascidella 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°16.208'N, 140°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 µm, 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-Profilier 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 *aspersa* were surveyed through four generations, the 2010, 2011, 2012, and 2013 cohorts.



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

158

159

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/m<sup>3</sup>) 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 µg/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 *A. aspersa* 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. *Asciidiella 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 *Asciidiella aspersa* with eggs and sperm in the ducts were found as late as September 2010, 2011,  
219 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. *Asciidiella 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. *Asciidiella 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. *Ascidella 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  
279 layer from spring to summer, and a strong seasonal thermocline is formed (Ohtani *et al.*, 1971a). The

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 *et al.* 2007; Carman



300 *et al.* 2010). The tsunami and the removal of damaged facilities may explain why the numbers of *A.*  
301 *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.*

320 *aspersa* (Global Invasive Species Database, 2010), and the description in the literature and many  
321 databases are based on this significant work (e.g. Global Invasive Species Database, 2010;  
322 Mackenzie, 2011). Millar (1952) studied the reproductive cycle and population dynamics of *A.*  
323 *aspersa* throughout 1950 and 1951 in Ardrossan, southwestern Scotland, which is their native habitat  
324 and we summarise his findings here.

325 Larvae settle in the summer (July–August) and grow until the end of September. *Ascidella*  
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. *Ascidella*  
328 *aspersa* have only one spawning season, and that is in the year after *A. aspersa* settled as larvae.  
329 *Ascidella 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–  
336 October) in Funka Bay is longer than the recruitment season in Ardrossan (July–August). *Ascidella*  
337 *aspersa* grow well until February in Funka Bay, and the average water temperature at 5–15-m depth  
338 fluctuates between 4 °C and 21 °C from July to February. In Ardrossan, *A. aspersa* grow until late in  
339 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. *Asciidiella 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

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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386

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428 [rn=&hci=-1&ei=-1&lang=EN&x=20&y=10](http://www.issg.org/database/species/search.asp?sts=sss&st=sss&fr=1&sn=Ascidiella+aspersa&rn=&hci=-1&ei=-1&lang=EN&x=20&y=10)

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433 [att/tpc053000000080h.pdf](http://www.fishexp.hro.or.jp/cont/hakodate/section/zoushoku/tpc05300000007ut-att/tpc053000000080h.pdf)

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536

537 FIGURE LEGENDS

538

539 **Fig. 1.** Cultured scallops, *Mizuhopecten yessoensis*, overgrown by the invasive ascidian, *Ascidella*  
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°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 *Ascidella aspersa*.

549

550 **Fig. 3.** Seasonal variation in larval density of *Ascidella 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,

557 May.

558

559 **Fig. 5.** Seasonal variation in the number of *Ascidella 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 *A. aspersa* 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 *Ascidella aspersa* on cultured scallops at all depths.

568

569 **Fig. 7.** Seasonal variation in the body length of *Ascidella 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 *Ascidella 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).

577 Average and standard error of wet weight (w.w. in grams [g]) of *A. aspersa* per month at each depth  
578 is shown: (A) 2010 cohort from July 2010 to May 2011; (B) 2011 cohort from June 2011 to June  
579 2012; (C) 2012 cohort from June 2012 to June 2013; and (D) 2013 cohort from June 2013 to June  
580 2014. For June 2012 and 2013, cultured scallops hung in the previous year and the year were  
581 collected. Scales of vertical axes are different.

582

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

590

591 **Fig. 10.** Size frequency and the presence of sperm and eggs in the ducts of *Ascidella aspersa*: (A)  
592 2010 cohort; (B) 2011 cohort; and (C) 2012 cohort. Ascidiens having neither eggs nor sperm in their  
593 ducts are regarded as immature.

594

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

597 size ( $M_{50}$ ) indicates the size at which 50% of *A. aspersa* mature, estimated according to the logistic  
598 curves.

599

600 **Fig. 12.** Life history of *Ascidella 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.



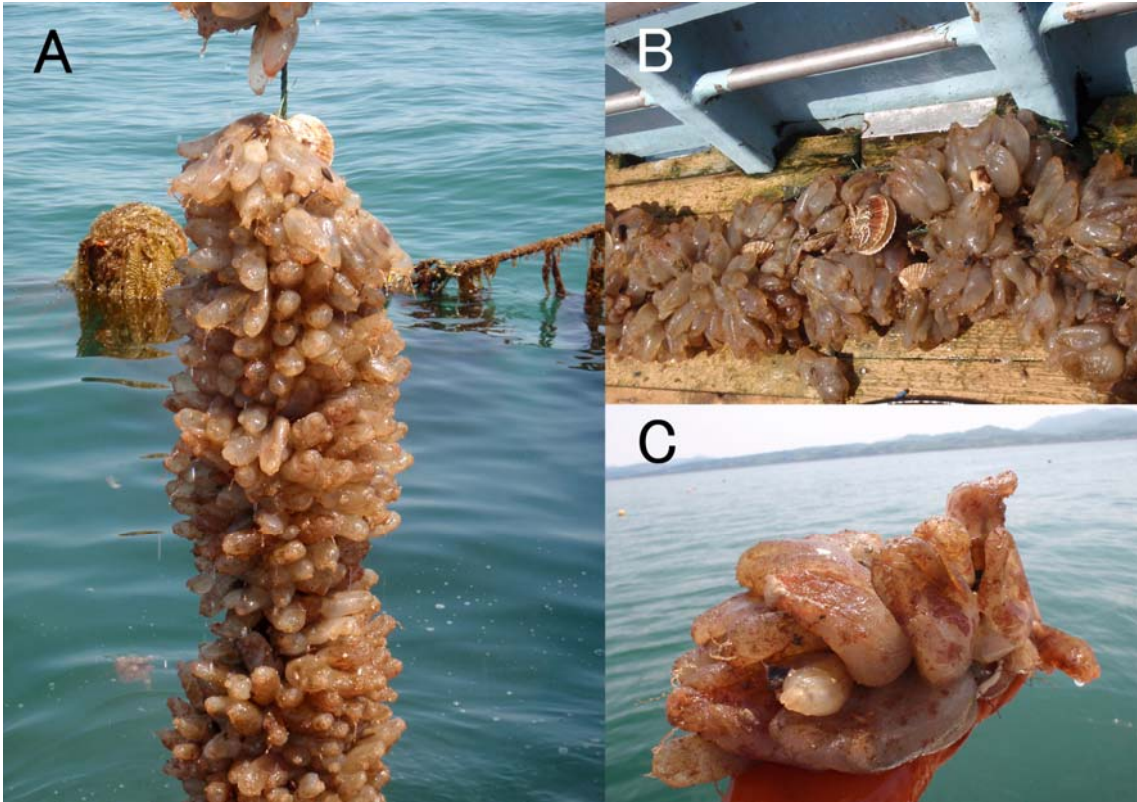


Fig.1

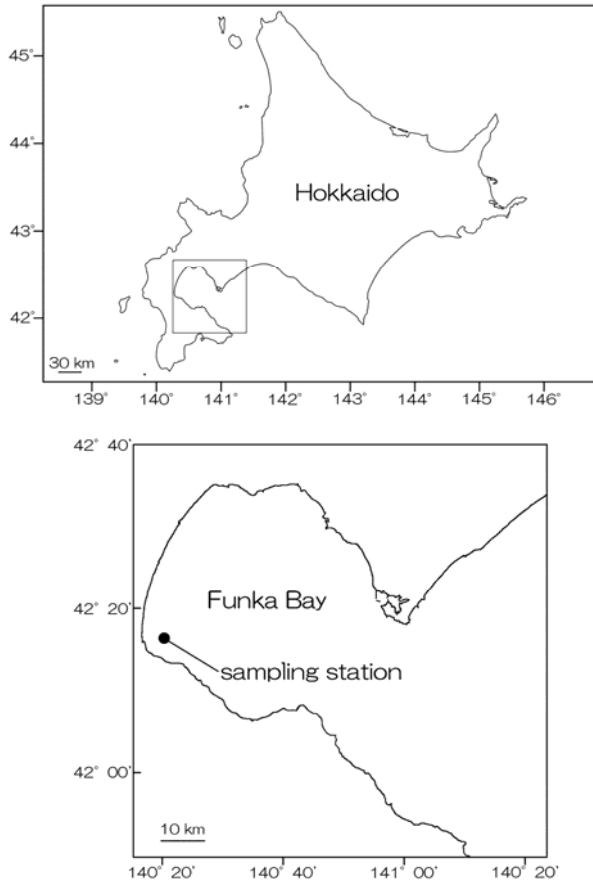


Fig.2

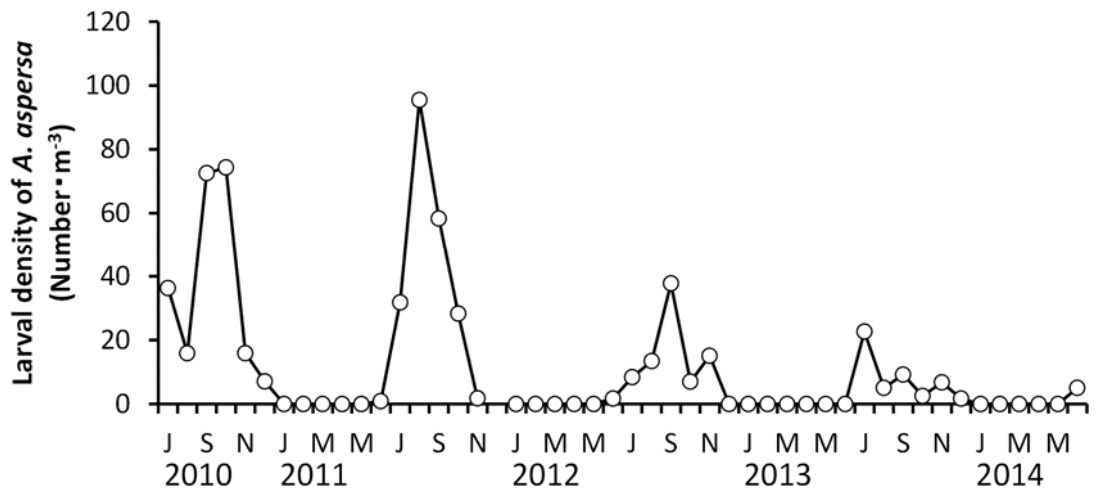


Fig.3

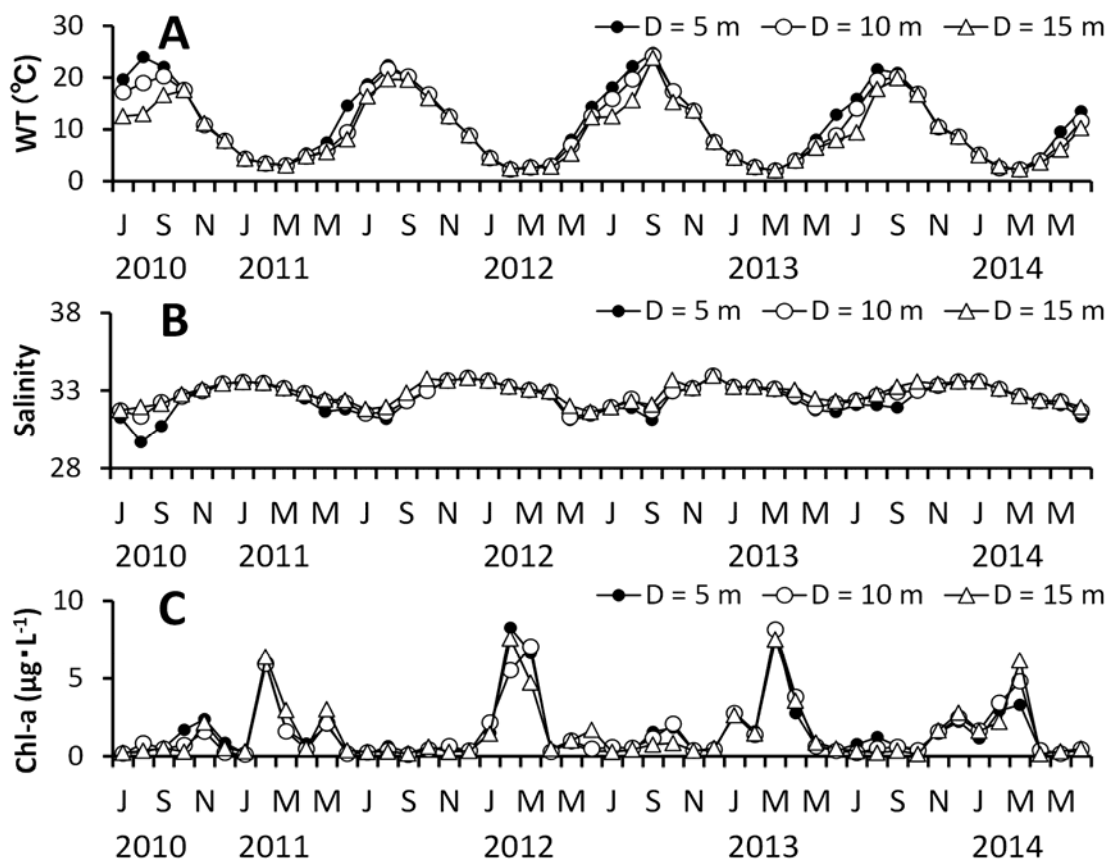


Fig.4

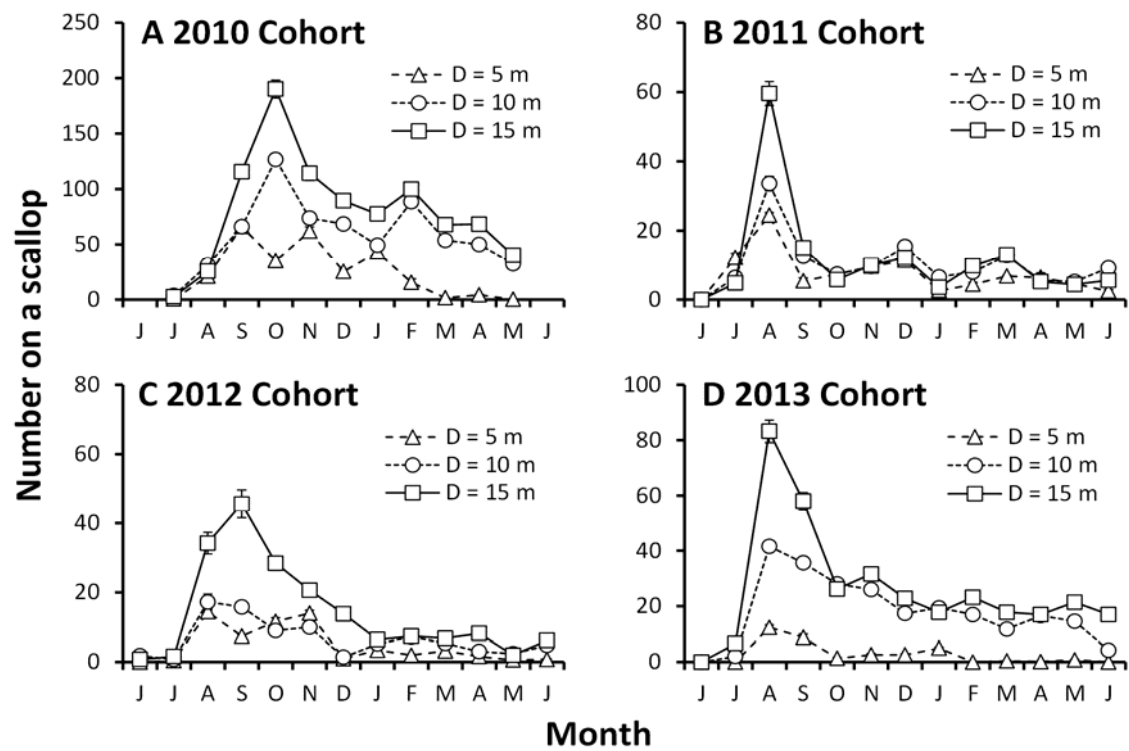


Fig.5

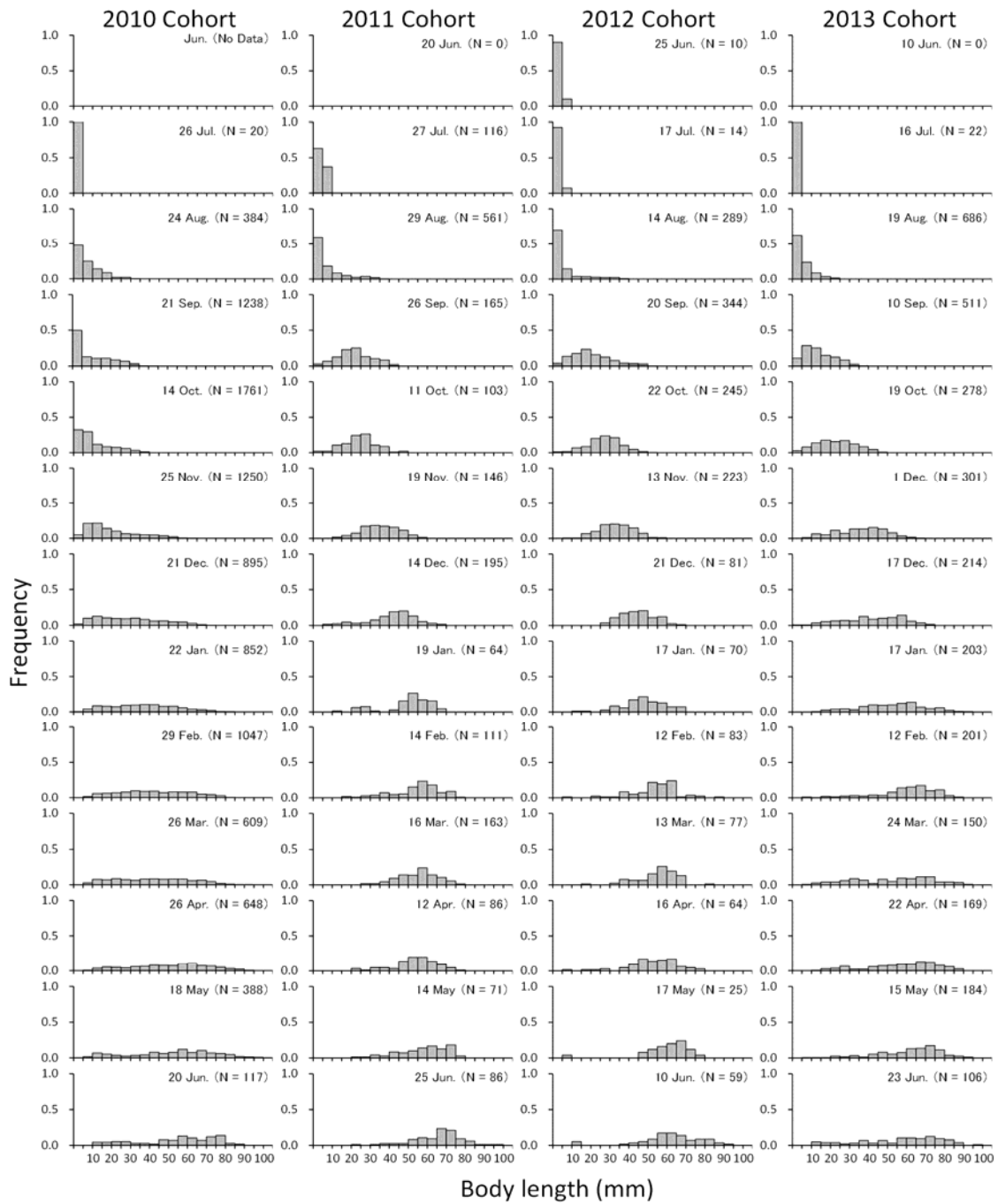


Fig.6

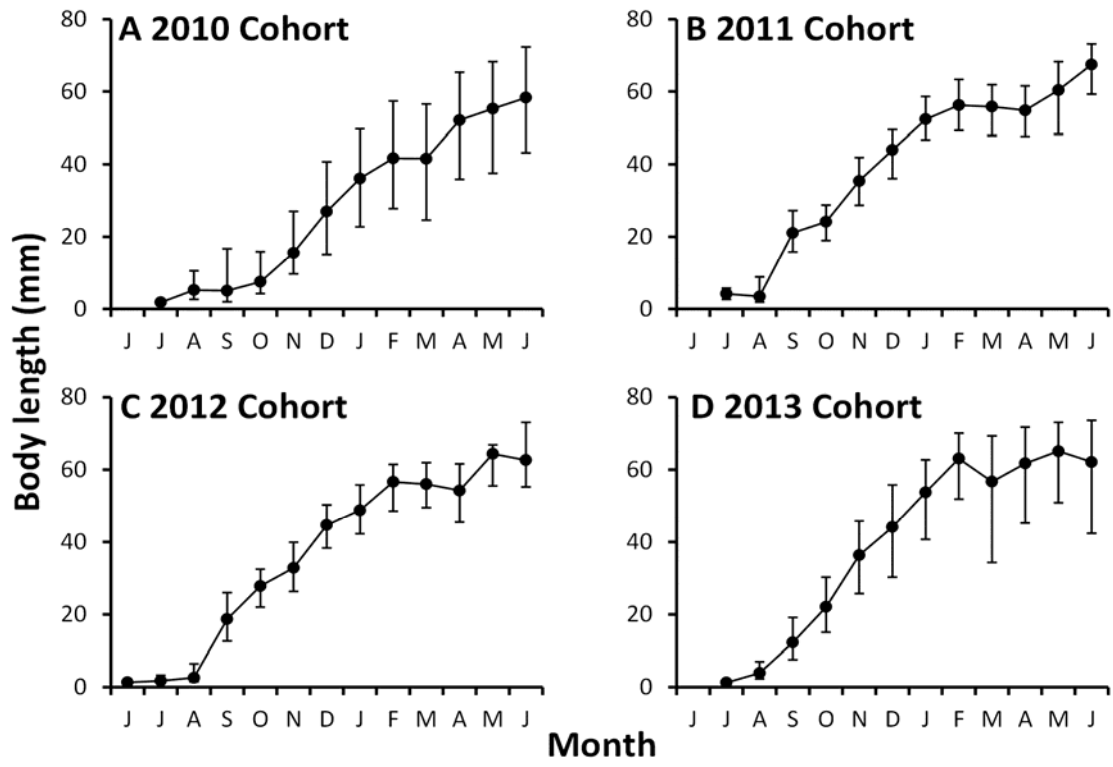


Fig.7

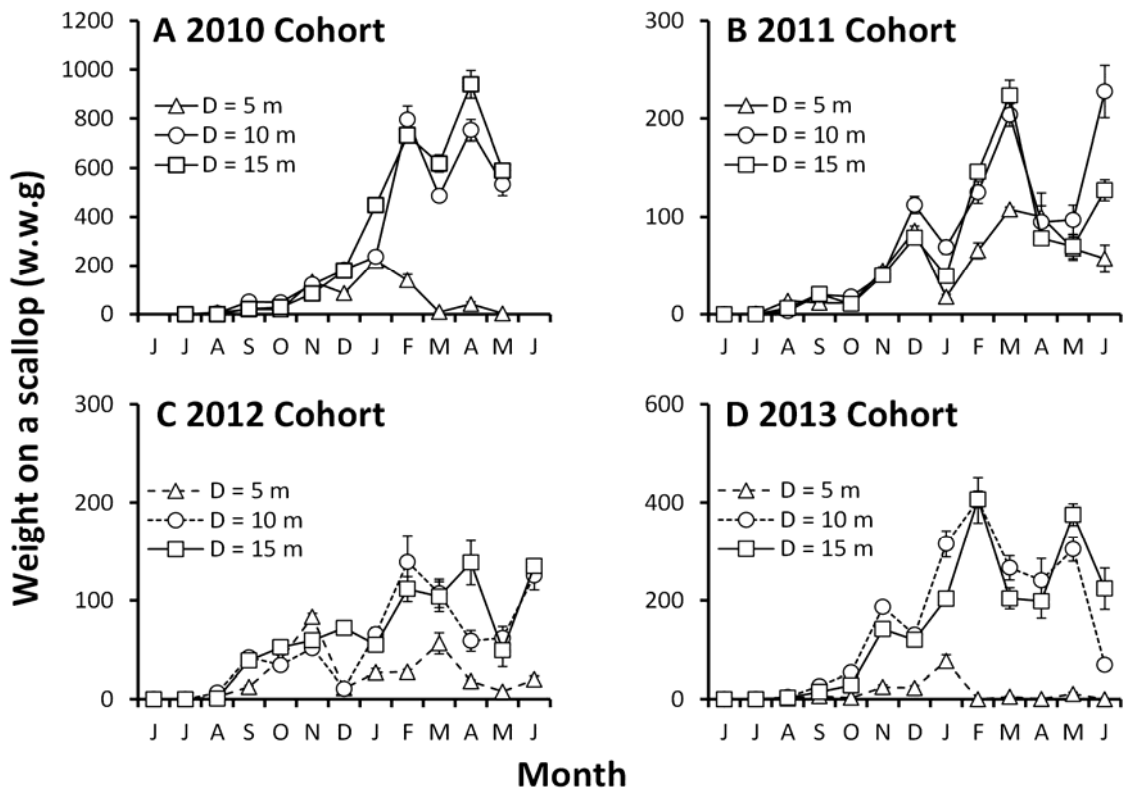


Fig.8



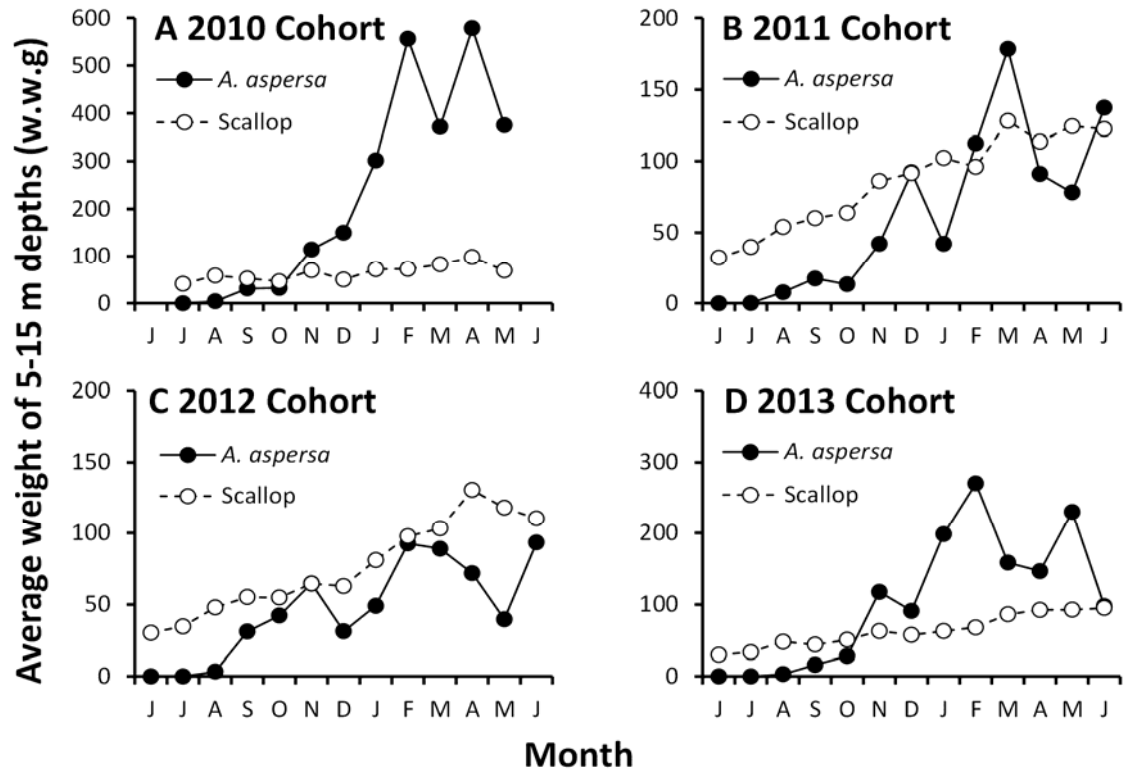


Fig.9

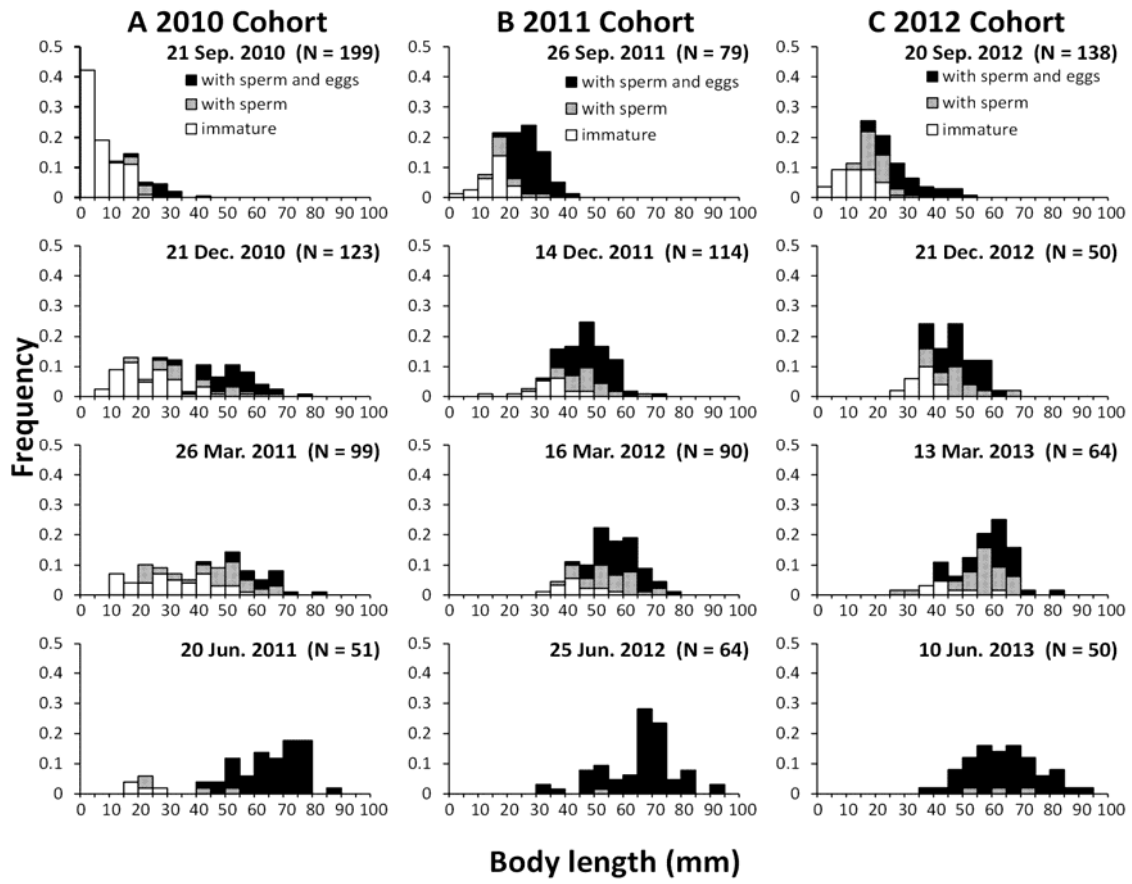


Fig.10

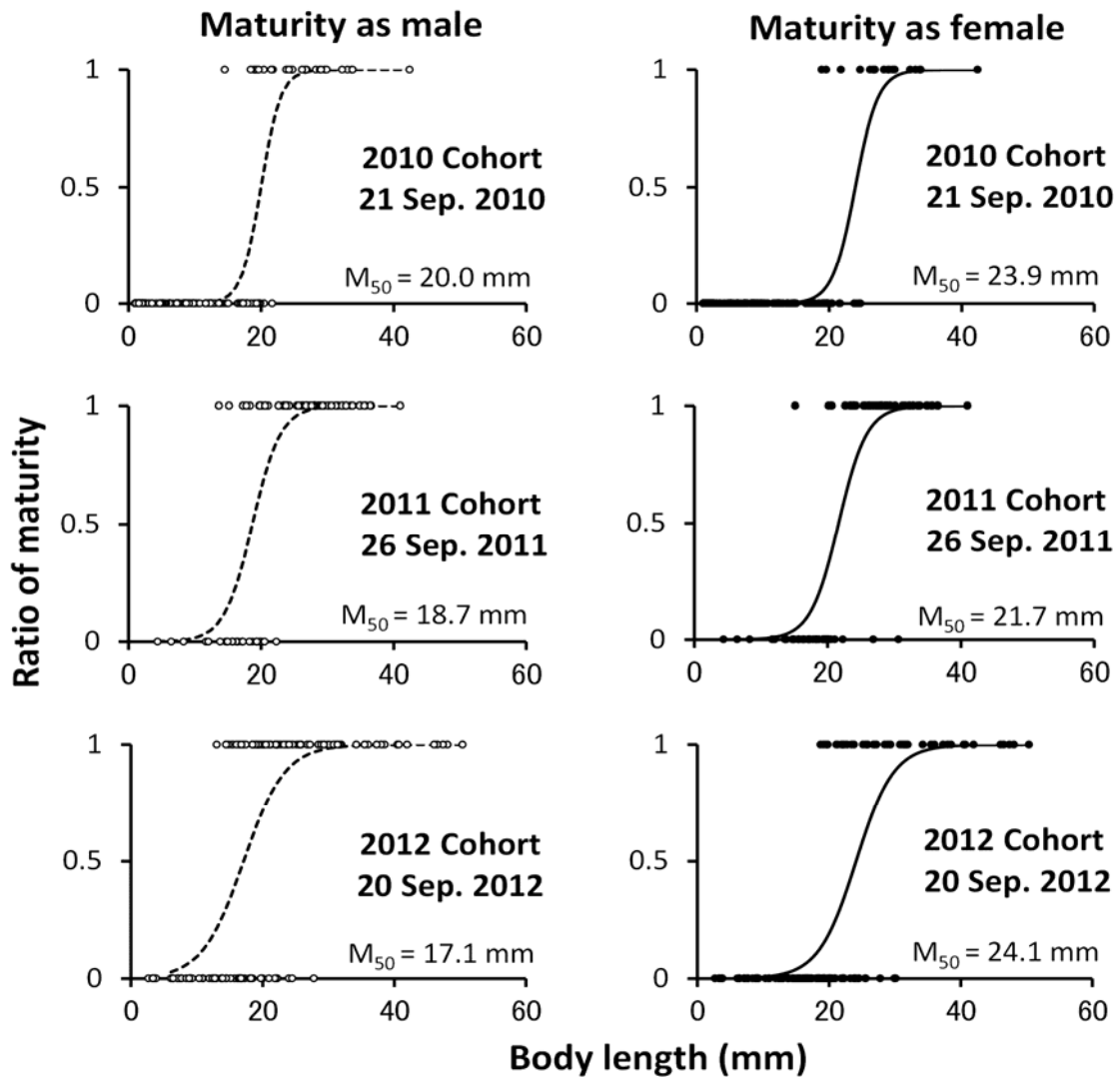


Fig.11

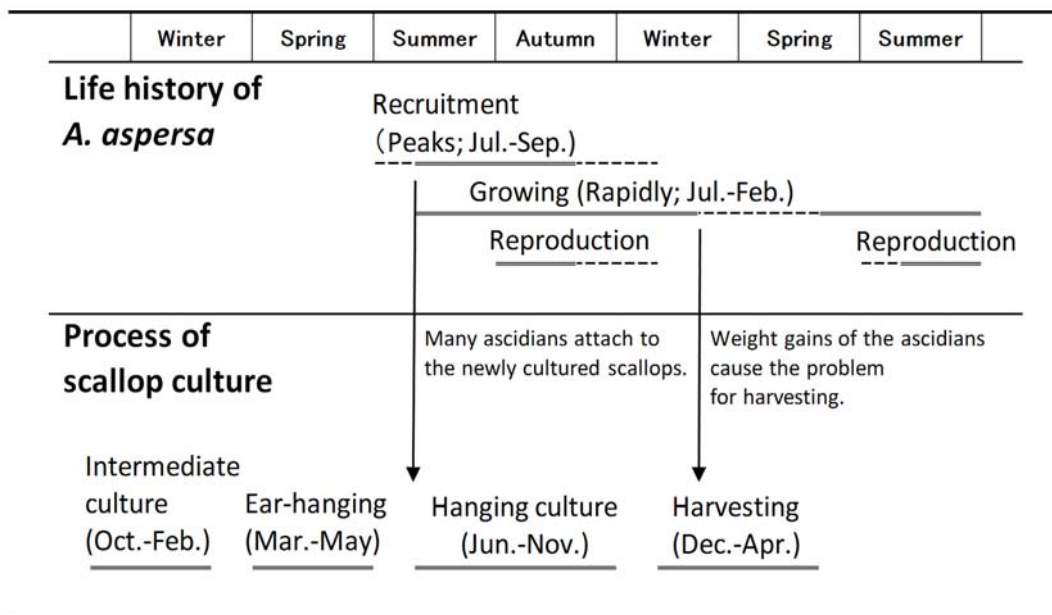


Fig.12

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

As male	Explanatory variable								50%Maturity size (mm) ( $-\beta_0/\beta_1$ )
	Intercept ( $\beta_0$ )				Body length ( $\beta_1$ )				
	Coef.	SE	z	p	Coef	SE	z	p	
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								Maturity size (mm) ( $-\beta_0/\beta_1$ )
	Intercept ( $\beta_0$ )				Body length ( $\beta_1$ )				
	Coef.	SE	z	p	Coef	SE	z	p	
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