Running Head: Life history traits of *Ascidiella aspersa* in Japan

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

Makoto Kanamori[a1, a2, c1], Katsuhisa Baba[a3], Masafumi Natsuike[a4] and Seiji Goshima[a2]

[a1] Hakodate Fisheries Research Institute, Fisheries Research Department, Hokkaido Research Organization, 20-5, Benten, Hakodate, Hokkaido 040-0051, Japan

[a2] Graduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido, 041-8611, Japan

[a3] Fisheries Research Department, Hokkaido Research Organization, 38, Hamamachi, Yoichi, Hokkaido 046-8555, Japan

[a4] Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro, Tokyo 152-8550, Japan

[c1]Correspondence should be addressed to: Makoto Kanamori, Hakodate Fisheries Research Institute, Fisheries Research Department, Hokkaido Research Organization, 20-5, Benten, Hakodate, Hokkaido 040-0051, Japan

email: kanamori-makoto@hro.or.jp

ABSTRACT

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

Keywords: invasive ascidian, *Ascidiella aspersa*, life history traits, population dynamics, aquaculture, 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).

However, no significant effects of invasive ascidians had been noted on the ecosystems or fisheries prior to the appearance of *Ascidiella aspersa* (Müller, 1776) (The Plankton Society of Japan and The Japanese Association of Benthology, 2009; Kanamori *et al*., 2012; Nishikawa *et al*., 2014).

The European sea squirt, *A. aspersa*, is a solitary marine and estuarine ascidian that is native from Norway to the Mediterranean (Berrill, 1950; de Kluijver & Ingalsuo, 2004; Mackenzie, 2011). The species has been introduced to North and South America, India, Australia, New Zealand, South Africa, South Korea, and Japan (Brewin, 1946; Kott, 1985; Nagabhushanam & Krishnamoorthy, 1992; Carlton, 2000; Robinson *et al*., 2004; Tatián *et al*., 2010; Kanamori *et al*., 2012; Pyo *et al*., 2012; Nishikawa *et al*., 2014). Because there are no efficient predators, *A. aspersa* can form large populations and subsequent high amounts of biomass, which redirects energy to decomposers and not to higher trophic communities (Currie *et al*., 1998). In addition, colonisation by *A. aspersa* reduces available substrata on which other species recruit successfully (Osman & Whitlatch, 2000). These characteristics have the potential to significantly affect species composition, reducing overall biodiversity (Mackenzie, 2011). *Ascidiella aspersa* also competes directly with other native filter-feeders, including economically important species such as scallops, mussels, and oysters (Currie *et al*., 1998). Therefore, *A. aspersa* is listed in the Global Invasive Species Database (2010), which is managed by the International Union for Conservation of Nature and Natural Resources, to increase awareness and to facilitate effective prevention and management activities.

The Japanese scallop, *Mizuhopecten yessoensis* (Jay, 1856), is one of the most important seafood 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 *asper*sa 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. asper*sa 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,

and 15 m at the sampling station. Each sample was filtered using a glass microfiber filter (GF/F, 47 mm, Whatman, GE Healthcare Life Science), and chlorophyll a (Chl-a) was extracted with 10 mL of *N*,*N*-dimethylformamide (DMF) (Wako Pure Chemical Industries, Ltd.). The Chl-a content was measured from the change using fluorescence (excitation 436 nm, emission 660 nm) before and after acidification by adding 0.1 mL of 5% HCl in 3 mL of the sample DMF solution. Fluorescence was measured by a fluorescence spectrophotometer (FP6300, JASCO Corp.). The concentration of Chl-a was calculated using Chl-a from chlorella (Wako Pure Chemical Industries, Ltd.) as the standard.

Sampling, measurement, and maturation level of *A. aspersa*

Five scallops, *Mizuhopecten yessoensis*, were collected monthly at 5-, 10-, and 15-m depths from a culture rope near the sampling station between July 2010 and June 2014 (total 15 scallops were collected monthly). In Funka Bay, scallops are produced from a natural population of larvae, from spring to summer. Scallops are reared in cages from autumn to spring, and this is called the intermediate culture. Juvenile scallops, after an intermediate culture, are suspended for hanging culture in spring. Collection of the scallops is initiated after spring (June or July) each year, and completed the following June (from July 2010 to June 2011, from June 2011 to June 2012, from June 2012 to June 2013, and from June 2013 to June 2014). When hanging cultures are started, *A. aspersa* are seldom found on the scallops, which means that the ascidians found on scallops after spring are newly settled. In this study, therefore, the life history traits and population dynamics of *A. aspersa* were surveyed through four generations, the 2010, 2011, 2012, and 2013 cohorts.

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

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^3) 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.

No clear variation in size structure of *A. aspersa* on cultured scallops was noted between depths. However, seasonal variation in the size frequency was noted when all depths were combined, as shown in Figure 6. Juvenile ascidians (body length < 5 mm) dominated during the period of increasing abundance. For the 2010 cohort, many juvenile ascidians were found from August to October, whereas for the 2011, 2012, and 2013 cohorts, juvenile ascidians were found mainly from July to August. Figure 7 shows the seasonal variation in the body length of *A. aspersa* on cultured scallops at all depths. *Ascidiella aspersa* grew well until February following each season, when their body length remained unchanged or decreased slightly from February to March or April.

The biomass of the scallops increased steadily in each year. The biomass of *A. aspersa* on scallops increased, with fluctuations, until February, and after that, changes were less clear (Figures 8, 9). For the 2010 cohort, the average weight of *A. aspersa* at all depths exceeded that of the scallops even in November, and was three to seven times heavier in harvest season, from December to April, meaning that the weight of *A. aspersa* accounted for 75–90% of the total weight of the harvest. For the 2011 cohort, the average weight of the ascidians was less than that of the scallops except in February and March. For the 2012 cohort, the average weight of the ascidians was always less than that of the scallops. The weight of the ascidians in the 2013 cohort was more than that of the scallops in and after November. June 2011 weight data are not represented because only five scallops were collected, without the depth information.

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
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. *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–
336 October) in Funka Bay is longer than the recruitment season in Ardrossan (July–August). *Ascidiella*
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. *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

length was from 10.3 to 44.6 mm (N = 30, examined by MK on 14 September 2015). The differences in method of fixation would not fully account for the disagreement of maturity size between Funka Bay and Ardrossan. Millar (1952) also described that ascidians in Loch Sween, Argyll, western Scotland, became mature at a smaller body size than did those in any of the samples from Ardrossan. Further analysis is required to determine whether maturity size is different between Funka Bay and native ranges.

As described above, compared with the native population in Ardrossan, *A. aspersa* in Funka Bay has a longer reproductive and growth period, earlier initiation of reproduction, and possibly smaller maturity size. The vigour and success of invasive species has been explained by favourable environments where they are introduced and by release from natural enemies and the adaptation or evolution of increasing competitive ability (Blossey & Nötzold, 1995, Keane & Crawley, 2002; Colautti *et al*., 2004). Further studies that assess environmental factors, such as temperature and food conditions, and enemies regulating the population in native regions, are necessary to compare life history traits of the global invasive species, *A. aspersa*, in native and introduced ranges.

379 ACKNOWLEDGEMENTS

380 We thank Professor Teruaki Nishikawa of Toho University for the invaluable information on
381 identification of ascidians. We are also grateful to Mr. Daisuke Achiya of the Yakumo Town
382 Fisheries Cooperative, Associate Professor Isao Kudo of the Hokkaido University, and the students
383 in Research Group of Marine Environmental Science, Graduate School of Fisheries Sciences,
384 Hokkaido University for their helpful assistance in the field samplings. We appreciate Dr. Joan
385 Cartes and the anonymous reviewer for their valuable suggestion and comments.

386

387 FINANCIAL SUPPORT

388 This study was conducted as a part of the contract researches from the Hokkaido Scallop Fisheries
389 Promotion Association in 2010, 2011, 2012, and 2013.

390

391

392

393

394

395

396

397

398 REFERENCES

399 **Berrill N.J.** (1950) *The tunicata with an account of the British species*. London: Ray Society.

400 **Blossey B. and Nötzold R.** (1995) Evolution of increased competitive ability in invasive
401 nonindigenous plants: a hypothesis. *Journal of Ecology* 83, 887–889.

402 **Bourque D., Davidson J., MacNair N.G., Arsenault G., LeBlanc A.R., Landry T. and Miron G.**
403 (2007) Reproduction and early life history of and invasive ascidian *Styela clava* Herdman in
404 Prince Edward Island, Canada. *Journal of Experimental Marine Biology and Ecology* 342, 78–84.

405 **Brewin B.I.** (1946) Ascidians in the vicinity of the Portobello Marine Biological Station, Otago
406 Harbour. *Transactions of the Royal Society of New Zealand* 76, 87–131.

407 **Carlton J.T.** (2000) Quo Vadimus Eotica Oceanica? Marine bioinvasion ecology in the twenty-first
408 century. In Pederson, J. (eds), *Marine Bioinvasions: Proceedings of the First National*
409 *Conference, January 24–27, 1999*. Cambridge: Massachusetts Institute of Technology, pp. 6–10.

410 **Carman M.R., Morris J.A., Karney R.C. and Grunden D.W.** (2010) An initial assessment of
411 native and invasive tunicates in shellfish aquaculture of the North American east coast. *Journal of*
412 *Applied Ichthyology* 26, 8–11.

413 **Colautti R.I., Ricciardi A., Grigorovich I.A. and Maclsaac H.J.** (2004) Is invasion success
414 explained by the enemy release hypothesis? *Ecology Letters* 7, 721–733.

415 **Currie D.R., McArthur M.A. and Cohen B.F.** (1998) Exotic marine pests in the port of Geelong,
416 Victoria. *Marine and Freshwater Resources Institute Report*, No.8, 57pp.

417 **Daigle R.M. and Herbinger C.M.** (2009) Ecological interactions between the vase tunicate (*Ciona*

418 *intestinalis*) and the farmed blue mussel (*Mytilus edulis*) in Nova Scotia, Canada. *Aquatic*
419 *Invasions* 4, 177–187.
420 **de Kluijver M.J. and Ingalsuo S.S.** (2004) *Ascidiella aspersa*, Macrobenthos of the North Sea-
421 Tunicata, Zoological Museum, University of Amsterdam. Available at:
422 http://wbd.etibioinformatics.nl/bis/tunicata.php?selected=beschrijving&menuentry=soorten&id=
423 17
424 **Dybern B.I.** (1969) Distribution and ecology of ascidian in Kviturdvikpollen and Vågsböpollen on
425 the west coast of Norway. *Sarsia*, 37, 21–40.
426 **Global Invasive Species Database.** (2010) *Ascidiella aspersa*. Available at:
427 http://www.issg.org/database/species/search.asp?sts=sss&st=sss&fr=1&sn=Ascidiella+aspersa&
428 rn=&hci=-1&ei=-1&lang=EN&x=20&y=10
429 **Goodbody I.** (2004) Diversity and distribution of ascidians (Tunicata) at Twin Cays, Belize. *Atoll*
430 *Research Bulletin* 524, 1–19.
431 **Hakodate Fisheries Research Institute.** (2010) 12th Scallop News in Funka Bay (2010). Available
432 at: http://www.fishexp.hro.or.jp/cont/hakodate/section/zoushoku/tpc05300000007ut-
433 att/tpc053000000080h.pdf
434 **Hokkaido Government.** (2012) Hokkaido Fisheries Today 2012. Sapporo: Hokkaido Government,
435 125pp.
436 **Howes S., Herbinger C.M., Darnell P. and Veraemer, B.** (2007) Spatial and temporal patterns of
437 recruitment of the tunicate *Ciona intestinalis* on a mussel farm in Nova Scotia, Canada. *Journal*

438 *of Experimental Marine Biology and Ecology* 342, 85–92.

439 **Imai I., Shimada H., Shinada A., Baba K., Kanamori M., Sato M., Kuwahara Y., Miyoshi K.,**

440 **Tada M., Hirano K., Miyazono A. and Itakura S.** (2014) Prediction of toxic algal bloom

441 occurrences and adaptation to toxic blooms to minimize economic loss to the scallop aquaculture

442 industry in Hokkaido, Japan. *PICES Scientific Report* 47, 7–16.

443 **Japan Meteorological Agency.** (2010a) The weather of summer (June–August). Available at:

444 http://www.jma.go.jp/jma/press/1009/01c/tenko100608.html.

445 **Japan Meteorological Agency.** (2010b) The weather of autumn (September–December). Available

446 at: http://www.jma.go.jp/jma/press/1012/01b/tenko100911.html.

447 **Japan Meteorological Agency.** (2012) Report on the 2011 off the Pacific coast of Tohoku

448 earthquake. *Technical report of the Japan Meteorological Agency*, 133, 479 pp.

449 **MAFF.** (2015) The 89th Statistical Yearbook of Ministry of Agriculture, Forestry and Fisheries

450 (2013–2014). Available at: http://www.maff.go.jp/e/tokei/kikaku/nenji_e/89nenji/index.html.

451 **Kanamori M., Baba K., Hasegawa N. and Nishikawa T.** (2012) Biological characteristics,

452 distinction and identification of *Ascidiella aspersa* (Müller, 1776), as an alien ascidian in northern

453 Japan (Technical Report). *Scientific Reports of Hokkaido Fisheries Research Institutes* 81, 151–

454 156.

455 **Kanamori M., Baba K., Konda Y. and Goshima S.** (2014) Distribution of the invasive ascidian

456 *Ascidiella aspersa* (Müller, 1776) (Urochordata, Ascidiacea) in Hokkaido, Japan. *Japanese*

457 *Journal of Benthology* 69, 23–31.

458 **Keane R.M. and Crawley M.J.** (2002) Exotic plant invasions and the enemy release hypothesis.
459 *Trends in Ecology and Evolution* 17, 164–170.
460 **Kosaka, Y. and Ito H. (**2006) Japan. In Shumway, S.E. and Parsons, G.J. (eds) *Scallops: Biology,*
461 *Ecology and Aquaculture*, 2nd edition. Tokyo: Elsevier, pp. 1093–1141.
462 **Kott P.** (1985) The Australian Ascidiacea Part I, Phlebobranchia and Stolidobranchia. *Memoirs of*
463 *The Queensland Museum*, 23: 1–440.
464 **Lambert G.** (2005) Ecology and natural history of the protochordates. *Canadian Journal of Zoology*
465 83, 34–50.
466 **Lambert G.** (2007) Invasive sea squirts: A growing global problem. *Journal of Experimental*
467 *Marine Biology and Ecology* 342, 3–4.
468 **Locke A. and Carman M.** (2009) An overview of the 2nd international sea squirt conference: What
469 we learned. *Aquatic Invasions* 4, 1–4.
470 **Mackenzie A.B.** (2011) Biological synopsis of the European sea squirt *Ascidiella aspersa. Canadian*
471 *Manuscript Report of Fisheries and Aquatic Sciences* 2968, 15 pp.
472 **Millar R.H.** (1952) The annual growth and reproductive cycle in four ascidians. *Journal of Marine*
473 *Biological Association of the United Kingdom* 31, 41–61.
474 **Millar R.H.** (1971) The biology of ascidians. *Advances in Marine Biology* 9, 1–100.
475 **Nagabhushanam A.K. and Krishnamoorthy P.** (1992) Occurrence and biology of the solitary
476 ascidian *Ascidiella aspersa* in Tamil Nadu coastal waters. *Journal of Marine Biological*
477 *Association of India* 34, 1–9.

Natsuike M., Kanamori M., Baba K., Moribe K., Yamaguchi A. and Imai I. (2014) Changes in abundances of *Alexandrium tamarense* resting cysts after the tsunami caused by Great East Japan Earthquake in Funka Bay, Hokkaido, Japan. *Harmful Algae* 39, 271–279.

Niermann-Kerkenberg E. and Hoffman D. K. (1989) Fertilization and normal development in *Ascidiella aspersa*（Tunicata）studied with Nomarski-optics. *Helgoländer Meeresuntersuchungen* 43, 245–258.

Nishikawa T., Kajiwara Y. and Kawamura K. (1993). Probable introduction of *Polyandrocarpa zorritensis* (Van Name) to Kitakyushu and Kochi, Japan. *Zoological Science* 10 (Supplement), 176.

Nishikawa T., Oohara I., Saitoh K., Shigenobu Y., Hasegawa N., Kanamori M., Baba K, Turon X. and Bishop J.D.D. (2014) Molecular and morphological discrimination between an invasive ascidian, *Ascidiella aspersa*, and its congener *A. scabra* (Urochordata: Ascidiacea). *Zoological Science* 31, 180–185.

Ohtani K., Akiba Y., Yoshida K. and Ohtuki T. (1971a) Studies on the change of the hydrographic conditions in the Funka Bay III. Oceanographic conditions of the Funka Bay occupied by the Oyashio water. *Bulletin of the faculty of Fisheries Hokkaido University* 22, 129–142.

Ohtani K., Akiba Y., Ito E. and Onoda M. (1971b) Studies on the change of the hydrographic conditions in the Funka Bay IV. Oceanographic conditions of the Funka Bay occupied by the Tsugaru Warm Waters. *Bulletin of the faculty of Fisheries Hokkaido University* 22, 221–230.

Osman R.W. and Whitlatch P.B. (2000). Ecological interactions of invading ascidians within

epifaunal communities of southern New England. In Pederson, J. (eds), *Marine Bioinvasions: Proceedings of the First National Conference, January 24–27, 1999*. Cambridge: Massachusetts Institute of Technology, pp.164–174.

Pyo J., Lee T. and Shin S. (2012) Two newly recorded invasive alien ascidians (Chordata, Tunicata, Ascidiacea) based on morphological and molecular phylogenetic analysis in Korea. *Zootaxa* 3368, 211–228.

R Development Core Team. (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www. R-project.org/.

Rius M. Carmen M. and Turon X. (2009) Population dynamics and life cycle of the introduced ascidian *Microcosmus squamiger* in the Mediterranean Sea. *Biological Invasions* 11, 2181–2194.

Robinson T.B., Griffiths C.L. and Kruger N. (2004) Distribution and status of marine invasive species in and bordering the West Coast National Park. *Koedoe* 47, 79–87.

Shenkar N. and Loya Y. (2008) The solitary ascidian *Herdmani momus*: native (Red Sea) versus non-indigenous (Mediterranean) populations. *Biological Invasions* 10, 1431–1439.

Shenkar N. and Loya Y. (2009) Non-indigenous ascidians (Chordata: Tunicata) along the Mediterranean coast of Israel. *Marine Biodiversity Records* 2, 1–7.

Shenkar N. and Swalla B.J. (2011) Global diversity of Ascidiacea. *PLoS ONE* 6, e20657.

Stachowicz J.J., Terwin J.R., Whitlatch R.B. and Osman R.W. (2002). Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Science of the United States of America* 99, 15497–

15500.

Svane I.B. and Young C.M. (1989) The ecology and behaviour of ascidian larvae. In Barnes, M. (ed.) *Oceanography and Marine Biology, An Annual Review 27*. Aberdeen : Aberdeen University Press, pp. 45–90.

Tatián M., Schwindt E., Lagger C. and Varela M.M. (2010) Colonization of Patagonian harbours (SW Atlantic) by an invasive sea squirt (Chordata, Ascidiacea). *Spixiana*, 33, 111–117.

The Plankton Society of Japan and The Japanese Association of Benthology. (2009) *Marine aliens introduced by human activities and their impacts on ecosystems and industries*. Hadano: Tokai University Press.

Tokioka T. and Kado Y. (1972) The occurrence of *Molgula manhattensis* (De Kay) in brackish water near Hiroshima, Japan. *Publications of the Seto Marine Biological Laboratory* 21, 21–29.

Vincente, C. S. and Sorbe J.C. (2013) Comparative life-histories, population dynamics and productivity of *Schistomysis* population (Crustacea, Mysida) in European shelf environments. *Journal of Sea Research* 81, 13–32.

Whitlatch R.B. and Bullard S.G. (2007) Introduction to the Proceedings of the 1st International Invasive Sea Squirt Conference. *Journal of Experimental Marine Biology and Ecology* 342, 1–2.

World Sea Temperatures. (2015) Saltcoats Sea Temperature. Available at: http://www.seatemperature. org/europe/united-kingdom/saltcoats.htm

537 FIGURE LEGENDS

538

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°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.

Fig. 5. Seasonal variation in the number of *Ascidiella aspersa* on cultured scallops. (first J on the horizontal axis is June of the year presented on the graph; last J is June of the following year). Average and standard error of number of *A. aspersa* on a scallop in each depth are 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.

Fig. 6. Seasonal variation in size frequency of *Ascidiella aspersa* on cultured scallops at all depths.

Fig. 7. Seasonal variation in the body length of *Ascidiella aspersa* on cultured scallops at all depths (first J on the horizontal axis is June of the year presented on the graph; last J is June of the following year). The medians are shown as representative values. Bars indicate 25th and 75th percentiles: (A) 2010 cohort from July 2010 to June 2011; (B) 2011 cohort from July 2011 to June 2012; (C) 2012 cohort from June 2012 to June 2013; and (D) 2013 cohort from July 2013 to June 2014.

Fig. 8. Seasonal variation in biomass of *Ascidiella aspersa* on cultured scallops (first J on the 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 *Ascidiella 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 *Ascidiella aspersa*: (A)
592 2010 cohort; (B) 2011 cohort; and (C) 2012 cohort. Ascidians having neither eggs nor sperm in their
593 ducts are regarded as immature.
594
595 **Fig. 11.** Relation between body length and maturity of *Ascidiella aspersa* in September. Maturity is
596 assessed by the presence of gametes in the ducts. The best-fit logistic curves are shown. Maturity

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

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

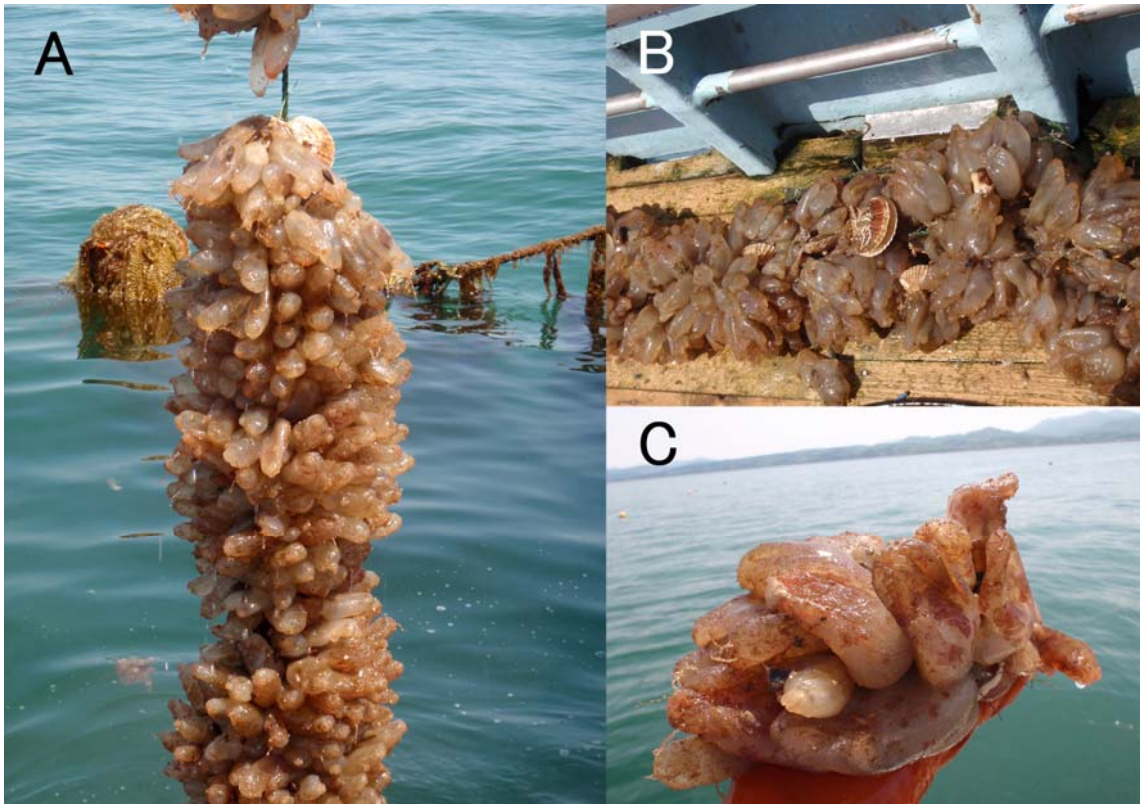
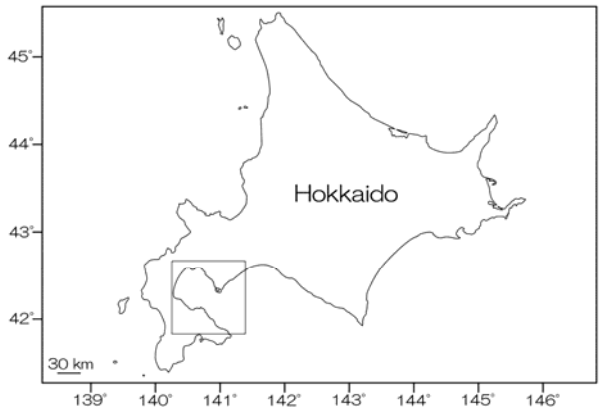


Fig.1


45°
44°
43°
42°
Hokkaido
30 km
139°
140°
141°
142°
143°
144°
145°
146°

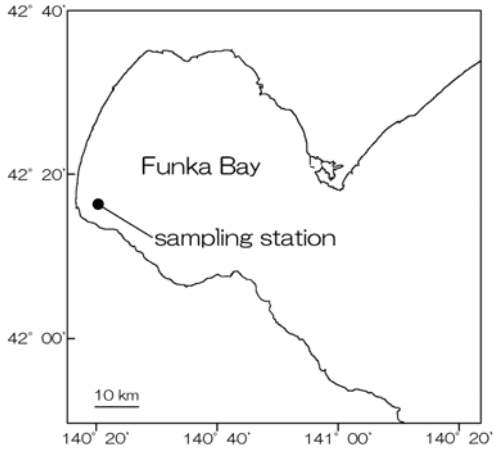

42° 40'
42° 20'
42° 00'
Funka Bay
sampling station
10 km
140° 20'
140° 40'
141° 00'
140° 20'

Fig.2

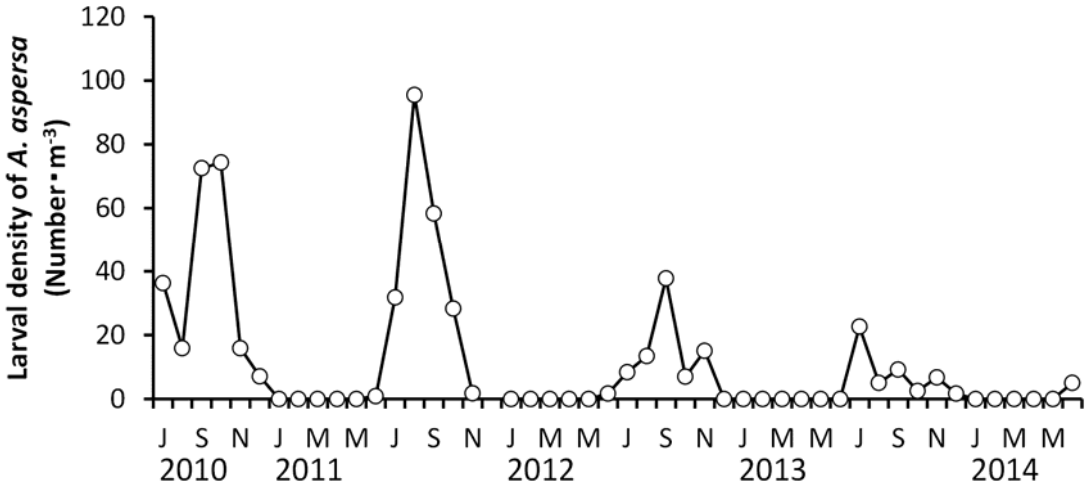

Larval density of A. aspersa
(Number・m-3)
120
100
80
60
40
20
0
J S N J M M J S N J M M J S N J M M J S N J M M
2010
2011
2012
2013
2014

Fig.3

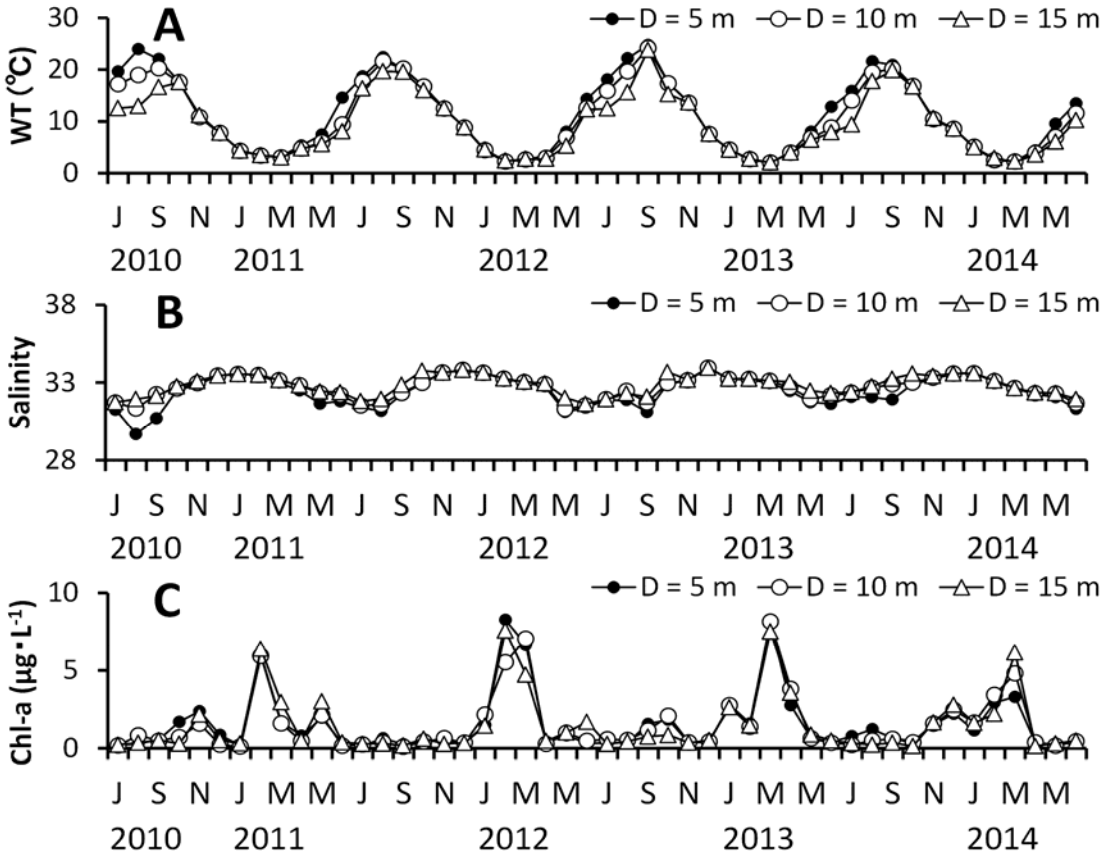

A
D = 5 m
D = 10 m
D = 15 m
WT (°C)
30
20
10
0
B
Salinity
38
33
28
C
Chl-a (μg·L-1)
10
5
0
J S N J M M J S N J M M J S N J M M J S N J M M
2010
2011
2012
2013
2014

Fig.4

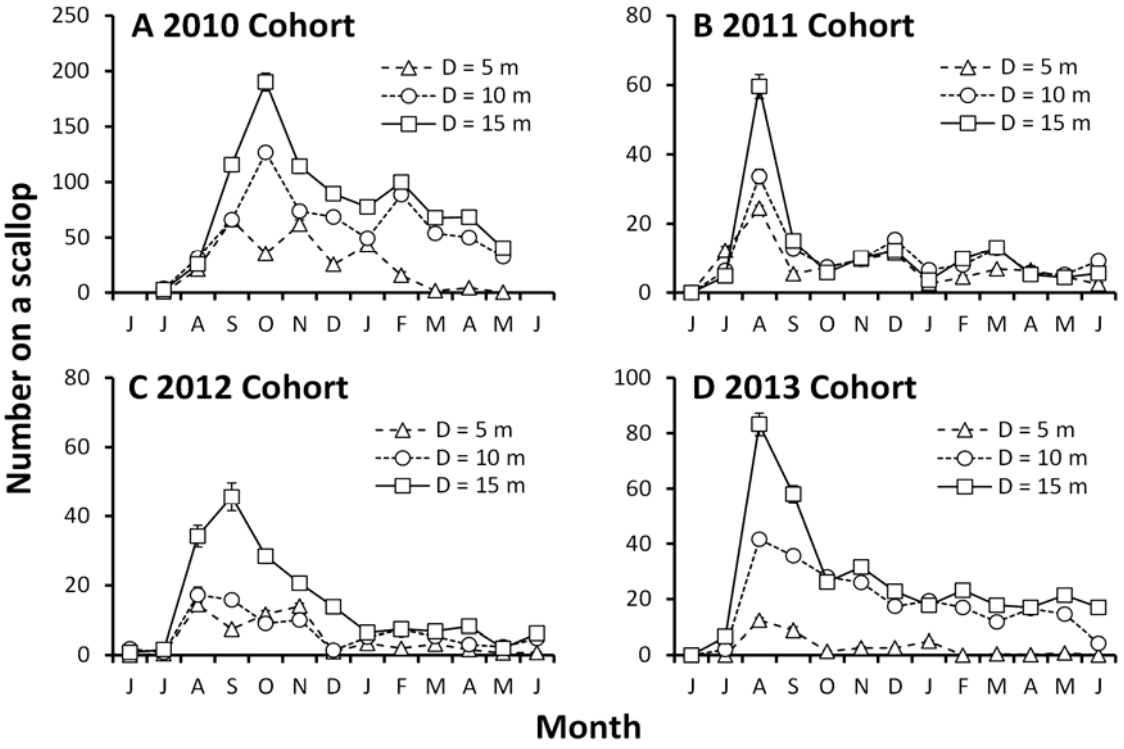

A 2010 Cohort
B 2011 Cohort
C 2012 Cohort
D 2013 Cohort
D = 5 m
D = 10 m
D = 15 m
Number on a scallop
Month
J J A S O N D J F M A M J

Fig.5

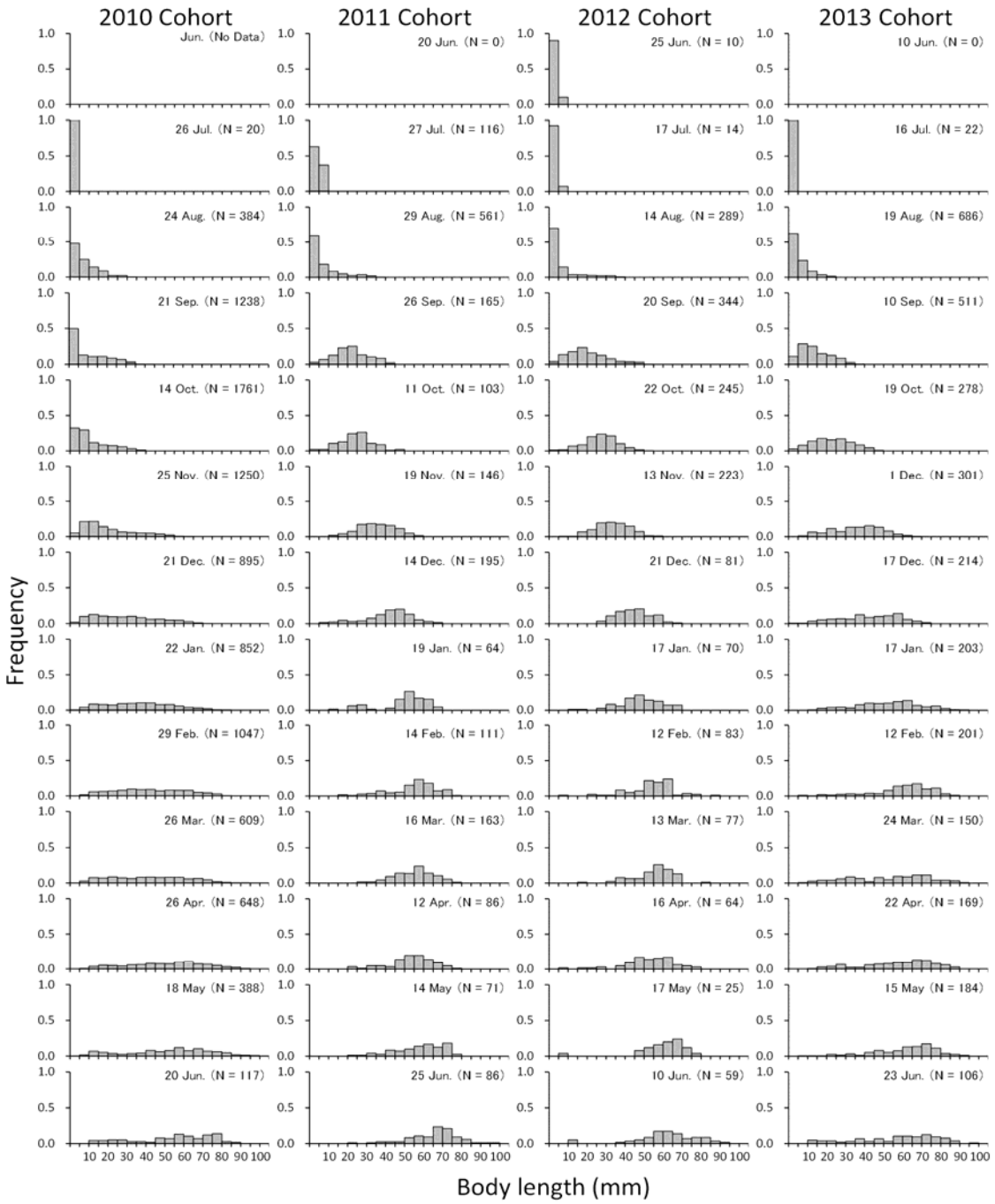

2010 Cohort
2011 Cohort
2012 Cohort
2013 Cohort
Jun. (No Data)
20 Jun. (N = 0)
25 Jun. (N = 10)
10 Jun. (N = 0)
26 Jul. (N = 20)
27 Jul. (N = 116)
17 Jul. (N = 14)
16 Jul. (N = 22)
24 Aug. (N = 384)
29 Aug. (N = 561)
14 Aug. (N = 289)
19 Aug. (N = 686)
21 Sep. (N = 1238)
26 Sep. (N = 165)
20 Sep. (N = 344)
10 Sep. (N = 511)
14 Oct. (N = 1761)
11 Oct. (N = 103)
22 Oct. (N = 245)
19 Oct. (N = 278)
25 Nov. (N = 1250)
19 Nov. (N = 146)
13 Nov. (N = 223)
1 Dec. (N = 301)
21 Dec. (N = 895)
14 Dec. (N = 195)
21 Dec. (N = 81)
17 Dec. (N = 214)
22 Jan. (N = 852)
19 Jan. (N = 64)
17 Jan. (N = 70)
17 Jan. (N = 203)
29 Feb. (N = 1047)
14 Feb. (N = 111)
12 Feb. (N = 83)
12 Feb. (N = 201)
26 Mar. (N = 609)
16 Mar. (N = 163)
13 Mar. (N = 77)
24 Mar. (N = 150)
26 Apr. (N = 648)
12 Apr. (N = 86)
16 Apr. (N = 64)
22 Apr. (N = 169)
18 May (N = 388)
14 May (N = 71)
17 May (N = 25)
15 May (N = 184)
20 Jun. (N = 117)
25 Jun. (N = 86)
10 Jun. (N = 59)
23 Jun. (N = 106)
Frequency
0.0
0.5
1.0
10 20 30 40 50 60 70 80 90 100
Body length (mm)

Fig.6

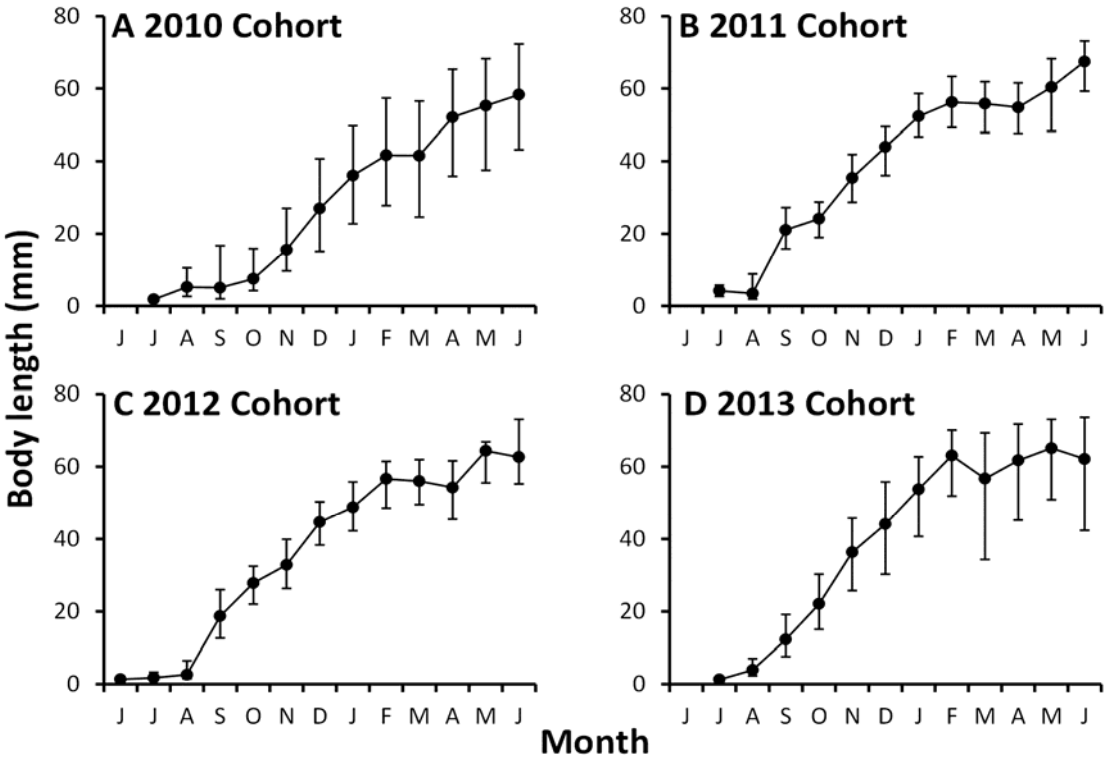

A 2010 Cohort
B 2011 Cohort
C 2012 Cohort
D 2013 Cohort
Body length (mm)
Month
0
20
40
60
80
J J A S O N D J F M A M J

Fig.7

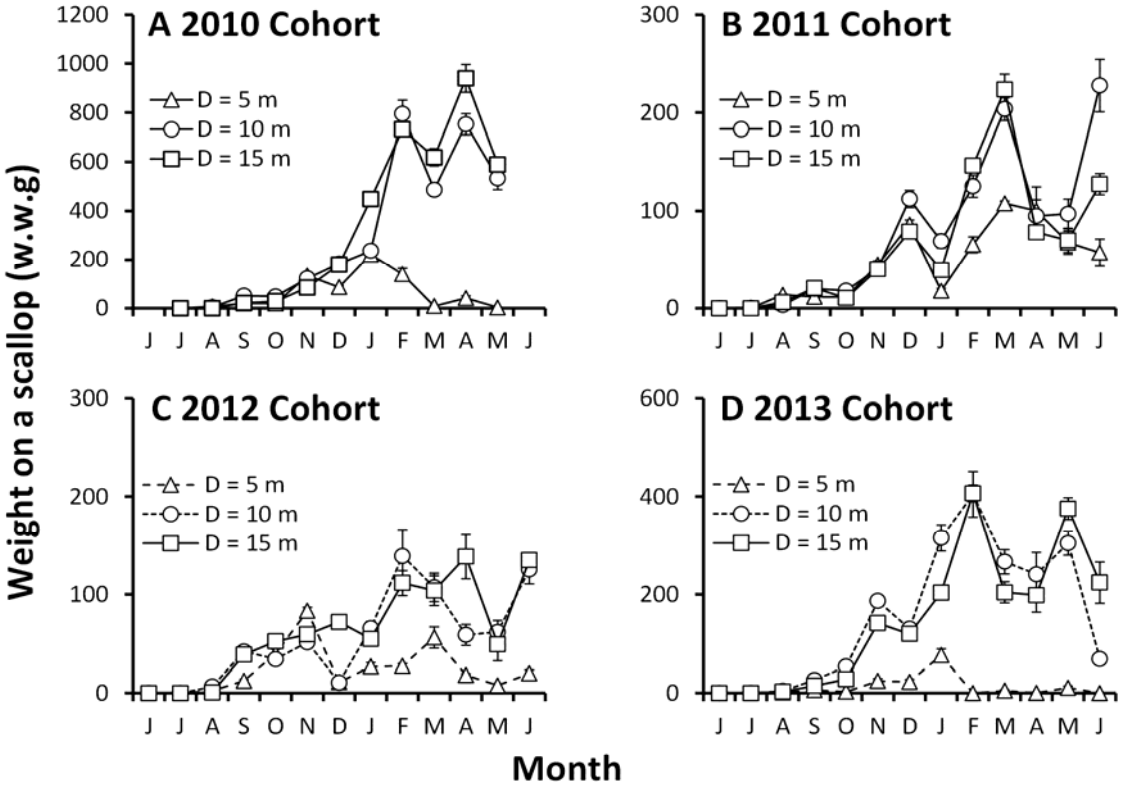

A 2010 Cohort
B 2011 Cohort
C 2012 Cohort
D 2013 Cohort
D = 5 m
D = 10 m
D = 15 m
Weight on a scallop (w.w.g)
Month
J J A S O N D J F M A M J

Fig.8

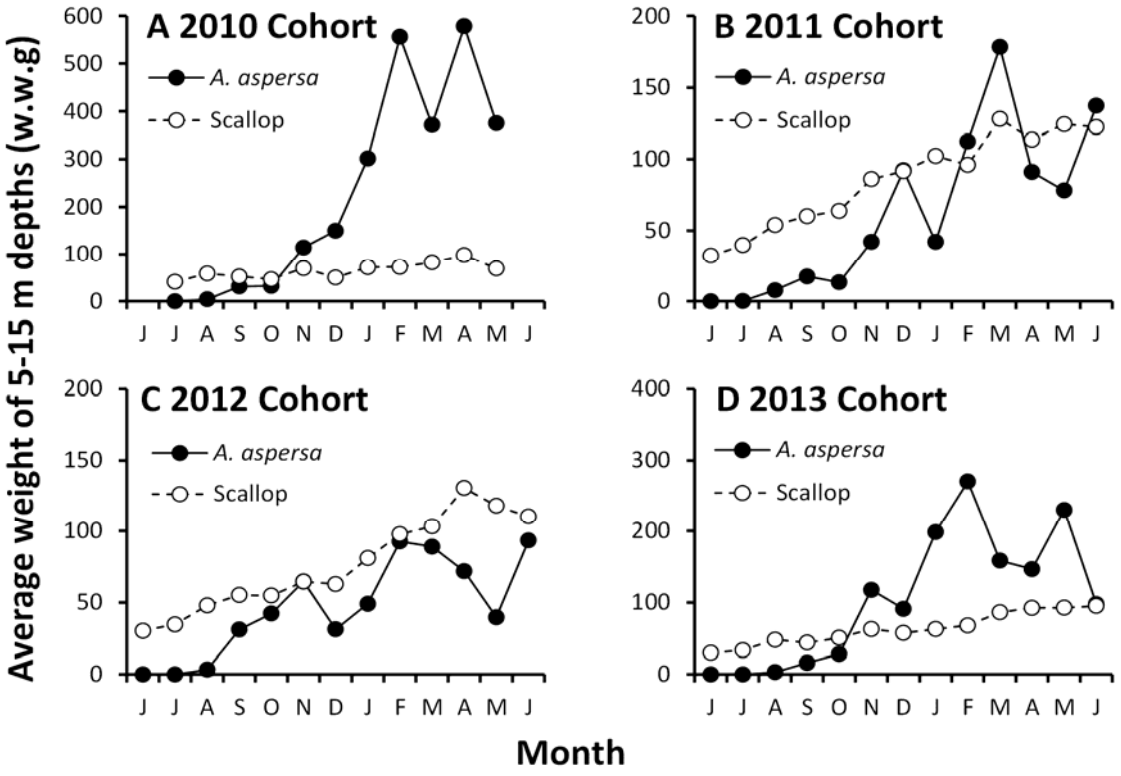

A 2010 Cohort
B 2011 Cohort
C 2012 Cohort
D 2013 Cohort
A. aspersa
Scallop
Average weight of 5-15 m depths (w.w.g)
Month
J J A S O N D J F M A M J

Fig.9

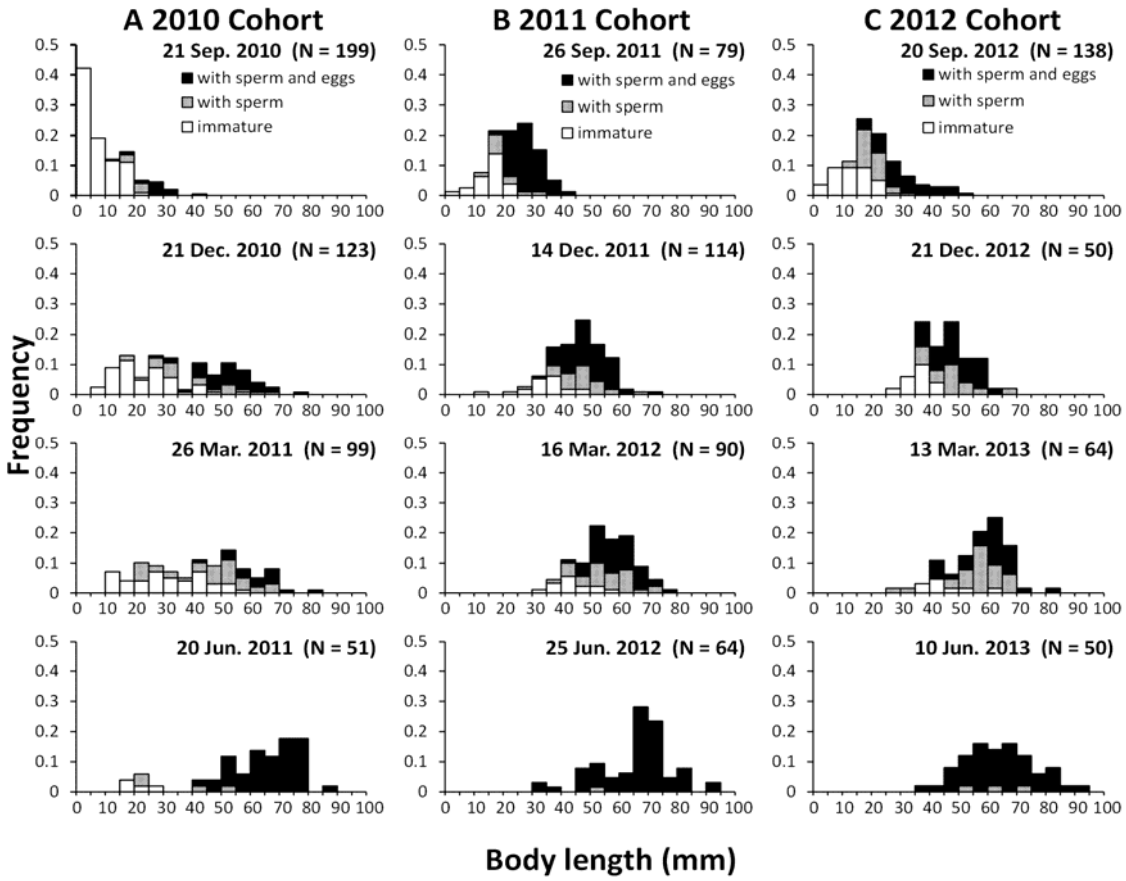

A 2010 Cohort
B 2011 Cohort
C 2012 Cohort
21 Sep. 2010 (N = 199)
26 Sep. 2011 (N = 79)
20 Sep. 2012 (N = 138)
with sperm and eggs
with sperm
immature
21 Dec. 2010 (N = 123)
14 Dec. 2011 (N = 114)
21 Dec. 2012 (N = 50)
26 Mar. 2011 (N = 99)
16 Mar. 2012 (N = 90)
13 Mar. 2013 (N = 64)
20 Jun. 2011 (N = 51)
25 Jun. 2012 (N = 64)
10 Jun. 2013 (N = 50)
Frequency
Body length (mm)

Fig.10

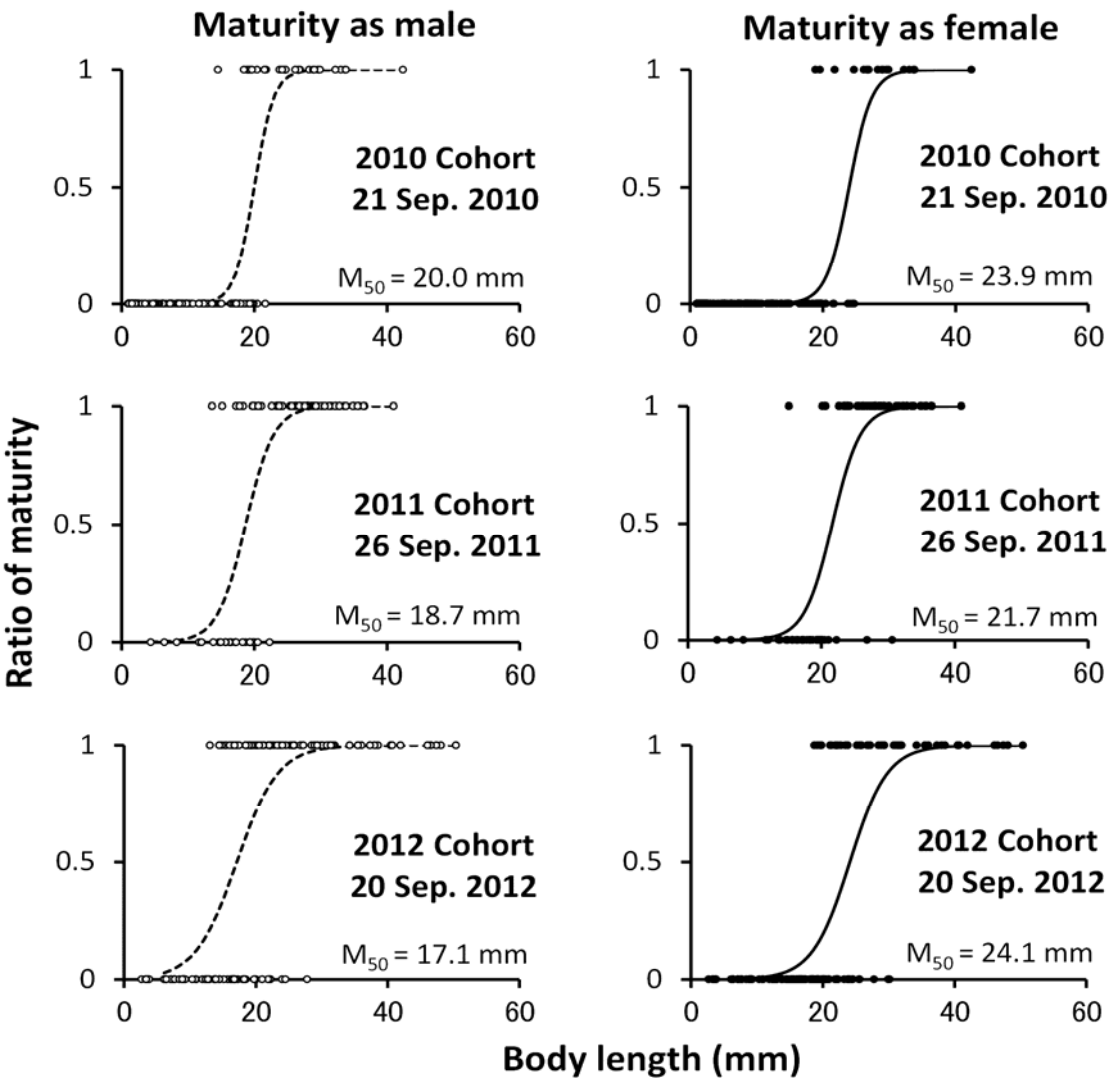

Maturity as male
Maturity as female
2010 Cohort
21 Sep. 2010
M50 = 20.0 mm
2010 Cohort
21 Sep. 2010
M50 = 23.9 mm
2011 Cohort
26 Sep. 2011
M50 = 18.7 mm
2011 Cohort
26 Sep. 2011
M50 = 21.7 mm
2012 Cohort
20 Sep. 2012
M50 = 17.1 mm
2012 Cohort
20 Sep. 2012
M50 = 24.1 mm
Ratio of maturity
Body length (mm)
0
0.5
1
0
20
40
60

Fig.11

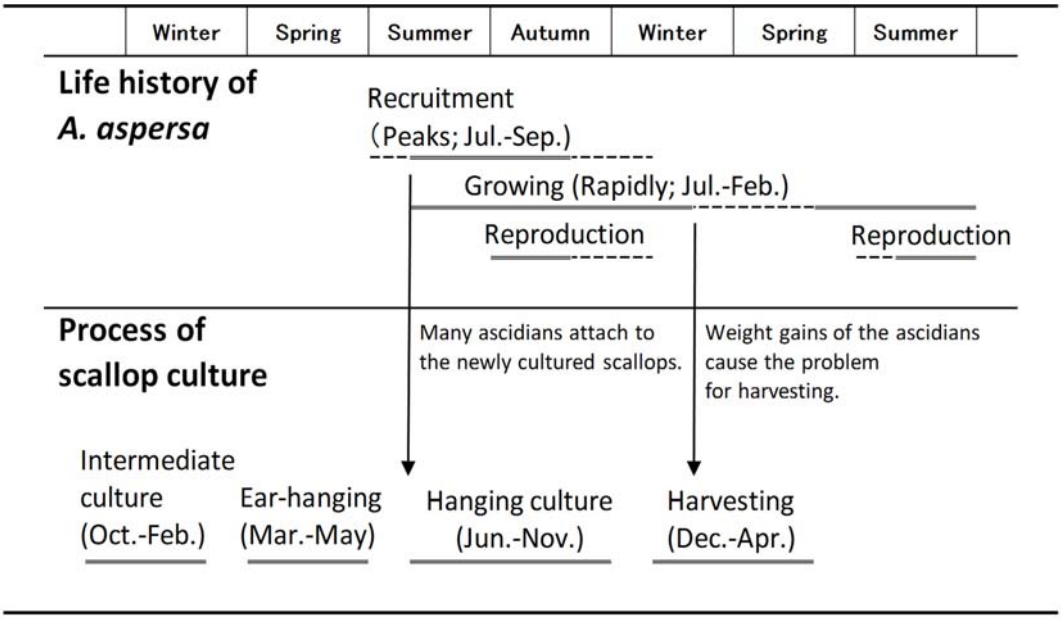

Winter
Spring
Summer
Autumn
Winter
Spring
Summer
Life history of
A. aspersa
Recruitment
(Peaks; Jul.-Sep.)
Growing (Rapidly; Jul.-Feb.)
Reproduction
Reproduction
Process of
scallop culture
Many ascidians attach to
the newly cultured scallops.
Weight gains of the ascidians
cause the problem
for harvesting.
Intermediate
culture
(Oct.-Feb.)
Ear-hanging
(Mar.-May)
Hanging culture
(Jun.-Nov.)
Harvesting
(Dec.-Apr.)

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 (β_0)				Body length (β_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 (β_0)				Body length (β_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