Effects of transplant sites and preventive measures against predation on the survival rates of pen shell in the Ariake Sea, Japan

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Received 11 December 2020; Accepted 28 May 2021 Responsible Editor: Shigeaki Kojima
doi: 10.3800/pbr.16.266

Abstract: In the Ariake Sea, Japan, pen shells Atrina spp. are an important fishery resource, but now abundance of the stock has decreased. Methods to increase the abundance include transplanting juvenile Atrina spp. in the sea and maintaining the survival rate high. We conducted transplant experiments to compare the survival rates of one genetic lineage of Atrina between 1) transplant sites and 2) preventive measures against predation. We conducted five runs of transplant of Atrina artificial seeds (about 10 cm in shell length) in the inner and outer sea areas, using cages with different mesh opening sizes. We found that 1) Atrina sp. survived better at stations in the outer sea area than in the inner sea area. This trend in the last run was partly explained by a heavy deposition of mud at a station in the inner sea area. 2) Atrina sp. survived better in cages with mesh opening size <20 mm than those with the size >20 mm. This was attributed to predatory animals, which presumably include the whelk Rapana venosa, octopi Octopodidae spp., and swimming crabs Charybdis spp. recorded by underwater time-lapse camera. In conclusion, transplant to the outer sea area and protection from predators with a mesh <20 mm opening are promising to increase the survival rate of one genetic lineage of Atrina transplanted in the Ariake Sea.

Key words: artificial seeds, Atrina pectinata Lineage 2, outer sea area, prevention of predation, survival

Introduction

Various species of commercially important bivalves have previously been transplanted to recover depleted stocks: e.g., Crassostrea virginica (Gmelin, 1791) in Chesapeake Bay (Lipcius et al. 2015); Tridacna spp. in southeast Asia (Mingoa-Licuanan & Gomez 2007); Rudites philippinarum (A. Adams & Reeve, 1850) in Mikawa Bay (Toba 2017). Some of the transplants have successfully led to increased catch through the harvest of the transplanted individuals after their growth and through the reproduction of the transplanted individuals (Lipcius et al. 2015; Toba 2017). Because one of the necessary conditions for a successful transplant is a high survival rate of the transplanted bivalves, previous transplant experiments of bivalves have examined various treatments leading to high survival rate (e.g. transplants to different sea areas, various equipment to protect transplanted bivalves, different body sizes of transplanted bivalves).

Some species in the family Pinnidae are also economically important and have been target species of transplant programs. Pinna nobilis Linnaeus, 1758 is a tourism resource in the Mediterranean Sea and has been transplanted to sustain the population (Bottari et al. 2017). Pinna bicolor Gmelin, 1791 is a fishery resource in the Indo-West Pacific and has been transplanted to increase the aquaculture production (Wu & Shin 1998). Atrina pectinata
Lineages 1 and 2 (sensu Liu et al. 2011) are an important fishery target species in the Ariake Sea, Japan (Koga & Aramaki 2013) and have been transplanted to revitalize the devastated fishery production (Ito 2017); note that these lineages are hereafter called just *Atrina* spp. owing to the taxonomic confusion (see Materials).

For *Atrina* spp. in the Ariake Sea various types of transplant experiments have been conducted since ca. 2000 (Ito 2017) to examine how to increase the survival rates. These experiments revealed higher survival rates for some transplant locations compared to other locations, namely, intertidal flats > the subtidal zone (Nasu et al. 2003; Yoshida et al. 2007); lower intertidal zones > higher intertidal zones (Iwanaga 2017); and hanging cages in the water column > the seabed (Ohashi & Tsukahara 2010; Matoba et al. 2016). Transplants starting from August, September or October resulted in higher survival rate than those from November or December (Iwanaga 2017). Large-sized specimens showed higher survival rates than small-sized specimens after transplant (Nasu et al. 2003; Yoshida et al. 2007). Protection of transplanted specimens with a cover net of 5 cm × 5 cm opening resulted in higher survival rates than with no protective measures owing presumably to prevention of predation by the longheaded eagle ray *Aetobatus narutobiei* White, Furumitsu & Yamaguchi, 2013 (Kawahara et al. 2004a, b).

These experiments to increase *Atrina* survival rates leave, however, two promising trials untested: one is a comparison between transplants to the inner sea area vs. the outer sea area of the Ariake Sea. Previously *Atrina* spp. have been transplanted to only the inner sea area including the fishery ground of *Atrina* spp. (Fig. 1) and have often shown low survival rates after transplant. Transplant to the outer sea area might lead to higher survival rates. The reason is that the outer sea area is likely to have better environmental conditions including higher dissolved oxygen concentration and more stable, higher salinity (Orita et al. 2015; Fisheries Research Agency et al. 2019; also see Nagasoe et al. 2020 and Kurihara et al. 2017 for the vulnerability of *Atrina* spp. to hypoxic and hyposaline water, respectively). In addition, in the outer area, patchy but high-density populations of adult *Atrina* spp. still occur (personal communication with the fishery cooperatives in Shimabara).

Another promising untested trial is the evaluation of the effect of preventive measures against the diverse predators that prey on *Atrina*. Such effects have been estimated for only the longheaded eagle ray *A. narutobiei* (Kawahara et al. 2004b; Fukumoto et al. 2017a). Preventive measures against diverse smaller predators might lead to higher survival rates of *Atrina* spp., because not only the ray but also many possible predators (e.g. predatory molluscs and crabs) occur in the Ariake Sea.

Considering the background, we examined two problems in the Ariake Sea. One is regarding whether *Atrina* sp. transplanted to the outer sea area survive better than those transplanted to the inner sea area. The other problem is regarding whether *Atrina* sp. survives better if...
it is protected from diverse predators including not only A. narutobiei but also smaller predators.

**Materials**

Aramaki (2013) reported the results of mtDNA COI region analyses that 97% of pen shells sampled from the Ariake Sea were *Atrina pectinata* Lineage 2 (sensu Liu et al. 2011) and the remaining 3% were *A. pectinata* Lineage 1. For the two lineages, orthodox species names are unclear owing to the taxonomic confusion of the genus *Atrina* (Lemer et al. 2014), and morphological differentiation is extremely difficult (Aramaki 2013; Hashimoto et al. 2018). In most of the previous studies, nevertheless, the two lineages in the Ariake Sea were identified only morphologically and thus appear to be confused. In the present study, we used artificial seeds of *A. pectinata* Lineage 1 that were identified by analyses of the mtDNA COI region. We acquired fertilized eggs generally in June in the laboratory from the adult *A. pectinata* Lineage 1 collected in the Ariake Sea. After these eggs developed into larvae and settled in laboratory tanks, they were reared in a downwelling system in the laboratory. Subsequently, from late August in general, they were reared in baskets suspended from a pontoon (32.80882°N, 129.76925°E) at 3 to 13 m depths in a protected bay. About one-year old seeds were used for the transplant experiments.

**Methods**

Transplant experiments were conducted in the inner and outer areas of the Ariake Sea for a total of five runs during 2017 to 2018 (Runs 1 to 5 in Table 1). Two stations were set in each of the inner area ("I1", "I2") and outer area ("O1", "O2"; Fig. 1) near which *Atrina* spp. had been found to occur (Comprehensive Survey Evaluation Committee 2017; personal communication with Shimabara Fishery Cooperative). Runs 1 to 5 addressed the first aim, namely, comparison of *Atrina* survival rate between stations I1 and I2 vs. O1 and O2 (Table 2). Runs 4 and 5 also addressed the second aim, namely, evaluation of the effect of protective measures against small predators on the *Atrina* survival rate (note: the term “small predators” hereafter denotes predatory animals smaller than the ray *Aetobatus narutobiei*). The methods common to all the five runs are as follows. Pen shells were transported from the pontoon to each station, using cooler boxes containing aerated seawater. To transplant these pen shells, divers set openable cages and nets more than 1 m apart from each other on the seabed

| Table 1. Protocols used in five runs of transplant experiments. |
|---------------------------------------------------------------|
| Stations | Run 1 | Run 2 | Run 3 | Run 4 | Run 5 |
| STations | I1, I2, O1, O2 | I2, O1 | I2, O1 | I2, O1 | I1, I2, O1, O2 |
| # pen shells transplanted into each equipment | 30 | 1 | 20 | 14 | 20 |
| Mean±SD shell length (mm) of pen shells transplanted | 104.3±2.3 | 116.0±13.8 | 96.9±16.6 | 96.9±16.6 | 93.9±8.5 |
| Equipment protecting pen shells | flat-wide cage (Fig. 2a) | pipe-shape net (Fig. 2b) | hat-shape net (Fig. 2c) | robust cage (Fig. 2d) of 10, 20, and 30 mm | robust cage (Fig. 2d) of "exposed type" and "protected type" |
| # replicates of equipment at each station | 4 | 12 | 2 | 1 for each opening | 3 for each of protected and exposed cages |
| Days when pen shells were transplanted | 31 May–10 June 2017 | 16–17 July 2017 | 18–19 August 2017 | 18–19 August 2017 * | 5–6 June 2018 |
| Periods from transplant to recapture (days) | 70–79 | 102 | 69 | 69 | 57 |

*: 30 August 2017 for 30-mm opening at I2  ▫: 57 days for 30-mm opening at I2

| Table 2. Stations for transplant experiments. Previous name indicates the name of each station in previous studies such as Fisheries Research Agency et al. (2020) |
|---------------------------------------------------------------|
| Station | Previous name | Latitude (°N) | Longitude (°E) | Mean depth (m) |
| I1 | P6 | 33.06250 | 130.22167 | 12.7 |
| I2 | T5 | 33.02933 | 130.36556 | 10.0 |
| O1 | M1 | 32.82721 | 130.36246 | 13.5 |
| O2 | KJ | 32.77361 | 130.37953 | 9.3 |
Transplant of pen shell

at each station. In these apparatuses, they dispersed the pen shells together with anthracites (i.e., grains of a hard coal) of a diameter of 2 mm so that the pen shells under the anthracites smoothly dig into the sea bed to fix themselves with the byssus (Kurihara, personal observation). In Run 2, exceptionally, divers embedded an individual pen shell in the sea bed inside each net. After the transplant, divers cleaned the cages and nets during neap tides every two weeks in general until recapturing the pen shells. After the recapture, alive pen shells were counted to calculate the survival rate for each cage and net.

The differences in methods among the five runs are as follows (Table 1). Cages and nets of various designs were used to preliminarily examine how to protect transplanted pen shells. In Run 1, "flat-wide cage" was used (Fig. 2a). The cage had an upper face of soft polyethylene net (opening: 16 mm × 16 mm), four sides of hard netlon net (22 mm × 21 mm), and a bottom without netting that was not embedded but put on the sea bed with stakes. In Run 2, "pipe-shape net" was used (Fig. 2b). It consisted of a polyvinyl chloride pipe (PVC pipe) on the lower side and a soft polyethylene net (opening: 16 mm × 16 mm) floated with a plastic float. This pipe was embedded in the sea bed with its lower limb as deep as 200 mm, namely the length of the pipe. In Run 3, "hat-shape net" was used (Fig. 2c). It was made of a soft polyethylene net (opening: 16 mm × 16 mm), the shape of which somewhat resembles a hat consisting of "crown" and "brim" parts. With plastic floats the crown part was floated, under which no net was set. The brim part was spread flat on the seabed and was embedded as deep as several centimeters with stakes of 20 cm length. In Runs 4 and 5, "robust cage" was used (Fig. 2d). The cage was made of either stainless or PVC mesh of a box shape with the bottom face without any mesh. For this cage, various mesh openings were prepared (10 to 115 mm; see below for detail). Half the height of the cages (15 cm) was embedded in the sediment. Pen shells were transplanted inside the flat-wide cages, the PVC pipe of the pipe-shape nets, and the robust cages; and under the crown part of hat-shape nets. Predators possibly access these pen shells from: 1) an interstice between the seabed and the bottom of the flat-wide cage, which is formed owing to deformation of the sea bed due to the tidal current; 2) the upper soft net of the flat-wide cage, pipe-shape net and hat-shape net, which predators might cut and/or bend; and 3) the opening of mesh of the robust cages, when the opening size is large.

In Run 1, the survival rate of pen shells was compared among the stations I1, I2, O1 and O2. Divers released 30 pen shells (mean±SD shell length: 104.3±2.3 mm) into each of four flat-wide cages (Fig. 2a) set at each station. The length of the period from the transplant to the recapture of pen shells differed among stations (70 to 79 days from 31 May–10 June to 18–19 August in 2017). Here, note that survival rates for longer durations tend to decrease,
because the survival rate during D days approximates to $S_i^0$, where $S_i$ denotes the survival rate per 1 day. Therefore, to eliminate such an effect of transplant duration, survival rates for each station and cage were calibrated as:

$$Sc_{ij} = \{S_i \ (1/D_i)\}^{76}$$

where $Sc_{ij}$ denotes the calibrated survival rate for station $i$ and cage $j$; $D_i$ denotes the transplant duration (unit: days) for station $i$; and $76$ is the approximate mean duration among stations. Then $Sc_{ij}$ is compared among stations with a mixed-effects logistic regression and logit link function (Crawley 2002) as:

$$\logit (E[Sc_{ij}]) = \mu + \beta_i + \varepsilon_{ij}$$

where $E[Sc_{ij}]$ denotes expected value of $Sc_{ij}$, $\mu$: intercept; $\beta_i$: effect of station $i$; $\varepsilon_{ij}$: normally distributed, random effect varying among cages within station $i$. The null hypothesis ($\beta_i=0$) was tested by an F test, considering overdispersion. The above and following analyses were performed with R ver. 3.6.2 (R Core Team 2019), and the logistic regression with the package "glmmML ver. 1.1.0" (Brostroem & Holmberg 2011).

In Run 2, the survival rate of pen shell was compared between the stations I2 and O1. An individual pen shell (mean±SD shell length: 116.0±13.8 mm) was transplanted into each of 12 pipe-shape nets for each station (Fig. 2b). The period from the transplant to the recapture of pen shells was 102 days for each station (16–17 July to 26–27 October 2017). Survival rates were compared between I2 and O1 with a Fisher's exact test.

In Run 3, the survival rate of pen shell was compared between the stations I2 and O1. Divers released 20 pen shells (mean±SD shell length: 96.9±16.6 mm) into each of two hat-shape nets at each station (Fig. 2c). The period from the transplant to the recapture of pen shells was 69 days for each station (18–19 August to 26–27 October 2017). Survival rates were compared between I2 and O1 with a mixed-effects logistic regression as in Run 1.

In Run 4, the survival rate was compared between the stations I2 and O1; and among the mesh opening sizes of cages to examine the effect of prevention of predation as in Run 4. Divers released 20 pen shells (mean±SD shell length: 93.9±8.5 mm) into each of six robust cages at each station (Fig. 3d). The six cages were divided into three “protected cages” and three “exposed cages”. The protected cages were the same as the cage used in Run 4, except that the opening of mesh was 15 mm. The exposed cages were made of hard resin with the same shape as the protected cage, and its lid with mesh opening 15 mm was exchangeable for another lid with mesh opening 115 mm. The opening 15 mm is likely to prevent the intrusion of small predatory animals (see Results for Run 4), and the opening 115 mm prevents the intrusion of the ray A. narutobiei (Usuki et al. 2012; Kumamoto Prefectural Fisheries Research Center 2017). On 5 to 6 June 2018 at each station, divers released pen shells into the protected and exposed cages all initially using a lid of 15-mm opening. On 21 to 22 June, divers exchanged the lids of the exposed cages from 15 mm to 115 mm opening at each station, after confirming that the pen shells had firmly fixed themselves in the sediment and thus were unlikely to be dispersed through the larger openings owing to the tidal current. Between the days of lid exchange and 16 to 21 October, divers counted surviving specimens once every two weeks. They judged a specimen alive if the specimen had firmly fixed itself in the sediment and/or closed the valves when touched. The survival rates estimated from the divers' count were compared with the true survival rates that were calculated from the correct numbers of living specimens recaptured from each cage at each station on the last surveys (16 to 19 October). This showed a high correlation between the divers' estimates and the true survival rates ($r=0.963, p=3.84·10^{-14}$). On 6 August at O1, one exposed cage and one protected cage were found to be tangled with a buoy rope and they were broken presumably owing to the strong tidal current. Therefore, for these two cages only
the divers’ counts of surviving pen shells before 6 August were used in the analysis.

In Run 5 survival rates were calculated from the number of living specimens recaptured (hereafter called “final survival rate” to differentiate from the survival rates estimated from the divers’ record). The final survival rates were calibrated for the mean experimental duration, 135 days, and were analyzed with a logistic regression similar to that for Run 1. The explanatory variables were the effects of station, cage type (i.e., exposed vs. protected cages), and their interaction. As the effect of station was found to be significant (see Results), multiple comparisons between stations with cage types pooled were done by a logistic regression with significance probabilities corrected by the Holm’s method (Rice 1989). For only Run 5, rather than the logit link function, the complementary log-log link function was used, because only the latter function lead to the convergence of parameters.

In addition to the foregoing analyses, survival rates were preliminarily compared among the four types of cages and nets (Fig. 1) used in 2017 (Runs 1 to 4 in Table 1), which possibly have different ability to protect pen shells from predators. Survival rates per 30 days, calculated as mentioned above ([survival rate for experimental period] \( / \) [transplant period]), were compared among the cages and nets for each of the stations I2 and O1 with no statistical test.

Environmental surveys

During all the runs of transplant experiments, water temperature (°C) and salinity were measured with a 10-min interval for each station by data loggers (Compact series and Infinity series of JFE Advantech Co., Ltd, http://ocean.jfe-advantech.co.jp/english/index.html) being set at 20 cm height above the sea bed. During Run 5, divers found that mud accumulated higher than the upper surface of the cages at I1 on 9 July 2018 owing presumably to rough weather (see Discussion), and thus they measured the thickness of mud for each cage and station after 6 August to compare the thickness among stations. In the center of each cage they used a graduated scale to measure the thickness of the sediment above the previous position of the seabed. This position was determined from the characteristics that the position was approximately 150 mm under the top face of the cage and that the sediment newly accumulated was softer than the sediment forming the previous seabed.

In Run 5, a time-lapse camera, TLC 200 Pro (Brinno Inc. https://www.brinno.com), protected in a waterproof case (height: 157 mm, diameter: 95 mm) was set near one of the exposed cages at each station to record predatory animals between the day of change of mesh opening and the next survey day. The distance between the lens and the side of the cage was set as 50 cm. The camera was run during 05:00 to 20:00 to utilize the available sunlight. It was set with a time interval of 20 seconds, focus 20 cm, frame rate 20 fps, white balance 2,800 inc, scene twilight, and HDR high. Photographs for 9 to 14 days were recorded for each station, depending on the battery life. We checked every photograph for each animal taxon to record how many photographs included possible predators. The number of photographs including a focal taxon was divided by the total number of photographs to calculate its relative frequency.

Results

Results 1: difference in Atrina survival rates between stations

Transplanted pen shells survived better at stations in the outer area of the bay than the inner area. In Run 1, although survival rates calibrated for the experimental periods did not significantly differ among stations, it exceeded zero at only O1 and O2 for some of the four replicate cages (0.071 per 76 days for one cage at O1, and 0.205 and 0.713 for two cages at O2); \( F = \text{Dev}_1 / \text{df}_1 / (\text{Dev}_2 / \text{df}_2) = 2.89/3 / (29.33/11) = 0.362, P = 0.782, \) logistic regression. In Run 2, although survival rates showed a marginally non-significant difference (\( P = 0.093, \) Fisher’s exact test), it was higher for O1 (0.33 for 102 days) than I2 (0.00). In Run 3, survival rate was significantly higher for O1 (survival rates for two nets during 69 days: 0.55 and 0.60) than I2 (0.00). In Run 3, survival rate was significantly higher for O1 (survival rates for two nets during 69 days: 0.55 and 0.60) than I2 (0.00). In Run 4, survival rates were higher for O1 than I2 for each of 10, 20, 30-mm openings (Fig. 3; no statistical test available).

In Run 5, the final survival rates calibrated for the mean experimental period, 135 days, significantly varied among stations (Table 3; \( \chi^2 = 30.99, \text{df} = 3, P = 8.55 \times 10^{-7}, \) logistic regression). The values showed no significant interaction between the effects of station and cage type (\( \chi^2 = 5.35, \text{df} = 3, P = 0.148 \)). For the protected cages the final survival rates

Table 3. Final survival rates in Run 5, calculated for the mean experimental duration, 135 days. Median (minimum–maximum) is presented for replicate cages.

| Station | Protected cage | Exposed cage |
|---------|----------------|--------------|
| I1      | 0.000 (0.000–0.000) | 0.000 (0.000–0.000) |
| I2      | 0.595 (0.545–0.949) | 0.000 (0.000–0.000) |
| O1      | 0.950 (0.900–1.000) | 0.000 (0.000–0.000) |
| O2      | 0.750 (0.400–0.750) | 0.050 (0.000–0.050) |
calibrated were lower in the order of I1 (median across replicate cages: 0.000 per 135 days) < I2 (0.595) < O2 (0.750) < O1 (0.950). For the exposed cages the values were lower for I1, I2, and O1 (median for each station: 0.000 per 135 days) than O2 (0.050). In multiple comparisons using the Holm’s correction, the final survival rates calibrated were significantly lower for I1 than the other stations ($\chi^2=11.08$ to 21.59, df=1, $P=3.4\cdot10^{-6}$ to 0.11)$^{-4}$).

Environmental factors during the experiments are presented in Table 4 and Fig. 4. Water temperature and salinity were similar among the four stations. The median of water temperature for each station was 24.5 to 24.8°C in 2017 (Runs 1 to 4) and 24.0 to 25.7°C in 2018 (Run 5). The median of salinity for each station was 30.9 to 31.9 in 2017 and 30.7 to 31.6 in 2018. The lowest salinity for each station was 25.3 to 27.7 in 2017 and 22.7 to 26.8 in 2018. In Run 5, median thickness of accumulated mud on the seabed was far greater at I1 (38 cm) than the other stations (0.5 to 5.0 cm; Fig. 4).

### Results 2: difference in *Atrina* survival rates between equipment for transplant

Pen shells survived better when transplanted into cages with smaller mesh openings in Runs 4 and 5. In Run 4, pen shells survived better in the order of 10-mm opening < 20-mm opening < 30-mm opening at I2 and O1 (Fig. 3; no statistical tests available). The difference was larger between 20- and 30-mm openings than between 10- and 20-mm openings for each station.

In Run 5, pen shells in general survived better in the protected cages than the exposed cages (Table 3). After the day of mesh opening alteration, the mean of the survival rates estimated from the divers’ records was higher for the protected cages than the exposed cages at each station except I1 (Fig. 5). The final survival rate calibrated for the mean experimental period (135 days) was significantly higher for the protected cages with a difference of 0.562

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**Table 4.** Water temperature and salinity at 20 cm height above the sea bed at stations I1, I2, O1 and O2.

| Year | Variable | Statistics | I1       | I2       | O1       | O2       |
|------|----------|------------|----------|----------|----------|----------|
|      |          |            | minimum  | minimum  | minimum  | minimum  |
| 2017 | temperature (°C) | 19.8 | 20.6 | 20.2 | 20.4 |
|      | 1st quartile | 23.3 | 23.5 | 23.1 | 23.6 |
|      | median     | 24.7 | 24.7 | 24.5 | 24.8 |
|      | 3rd quartile | 25.9 | 26.1 | 25.7 | 26.3 |
|      | maximum    | 28.3 | 28.8 | 28.0 | 29.3 |
|      | Salinity   | minimum  | 27.7 | 26.3 | 25.3 | 25.9 |
|      | 1st quartile | 30.2 | 30.5 | 31.5 | 29.7 |
|      | median     | 31.0 | 31.0 | 31.9 | 30.9 |
|      | 3rd quartile | 31.5 | 31.5 | 32.3 | 31.9 |
|      | maximum    | 32.5 | 32.3 | 33.1 | 33.0 |
| 2018 | temperature (°C) | 20.5 | 20.8 | 20.7 | 21.4 |
|      | 1st quartile | 22.5 | 23.7 | 23.2 | 23.6 |
|      | median     | 24.0 | 25.0 | 24.6 | 25.7 |
|      | 3rd quartile | 26.5 | 26.6 | 26.4 | 27.0 |
|      | maximum    | 28.2 | 29.4 | 29.1 | 29.1 |
|      | Salinity   | minimum  | 22.7 | 26.8 | 25.4 | 26.0 |
|      | 1st quartile | 29.7 | 30.2 | 31.1 | 30.6 |
|      | median     | 30.8 | 30.7 | 31.6 | 31.5 |
|      | 3rd quartile | 31.5 | 31.2 | 32.0 | 31.9 |
|      | maximum    | 32.2 | 32.1 | 32.7 | 32.6 |

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![Fig. 4. Boxplot of thickness of mud (cm) in Run 5. The bold center line denotes the median, and the upper and lower sides of the rectangle denote the third and first quartiles, respectively.](image-url)
than for the exposed cages ($\chi^2=35.98$, df=1, $P=2.00\times10^{-09}$, GLMM).

Animals recorded by a time-lapse camera at each station in Run 5 were categorized into 10 groups (Table 5). The groups included five animals possibly preying on the transplanted pen shells: the rapa whelk Rapana venosa (Valenciennes, 1846), octopi Octopodidae spp., swim-
ing crabs Charybdis spp. (mostly Charybdis japonica (A. Milne-Edwards, 1861)), a ray Batoidea sp. and a seabream Acanthopagrus schlegelii (Bleeker, 1854). Of these possible predators, R. venosa, Octopodidae spp., and Charybdis spp. showed relatively high occurrence rates (>1%) for some stations (Table 5) and were found inside the exposed cages (Divers' personal observations). Batoidea sp. was recorded only in one photograph at O1 (occurrence rate: 0.0044%), which showed the ray swimming over an exposed cage. Crustaceans showed a high occurrence rate at O1 (85.9%), because a majid crab, probably Pugettia quadridens (De Haan, 1839), remained on the side of the cage at O1. Asteroidea was recorded for O2, which was not categorized into a possible predator (see Discussion).

The stations O1 and I2 shared a trend that survival rates of pen shells per 30 days were highest for the robust cage and lowest for the flat-wide cage. At O1, they were higher in the order of robust cage (0.880)>hat-shape net (0.786)>pipe-shape net (0.724)>flat-wide cage (0.207). At I2, they were higher for the robust cage (0.743) than the other equipment (0.000).

**Discussion**

**Higher survival rate of pen shells transplanted to the stations in the outer area of Ariake Sea**

In general, Atrina sp. survived better when transplanted to the stations O1 and O2 than the stations I1 and I2. Of such differences between stations, the lower survival rate for I1 compared to O1 and O2 in Run 5 is likely to be due to the deposition of mud. Only at I1 mud accumulated and became thicker than the height of the cages for a long period including 9 July to 21 October. This should have caused death of the pen shells due to oxygen shortage, considering that pen shells of 1 year old (i.e. the age of our experimental specimens) mostly die within 4 days in sea water with dissolved oxygen concentration being experimentally regulated at <0.2 mg L$^{-1}$ (Nagase et al. 2020). Death due to sediment deposition has also been reported for scallop (Hendrick et al. 2016). Since pen shells at I1 died off not only in the exposed cages but also the protected cages, the major cause of the die off is not likely to be predation. The mud deposition at I1 is coincident with heavy rainfall (120.5 mm d$^{-1}$) and strong northerly wind (maximum speed: 11.4 m sec$^{-1}$) on 29 June 2018 (Japan Meteorological Agency 2020), which is considered to have caused erosion and transportation of mud from the tidal flat of the inner part of the Ariake Sea (Fisheries Research Agency et al. 2020). Such a heavy deposition of mud in the Ariake Sea as found in 2018 appears to have been very rare previously. The phenomena, however, might frequently happen in future because of recent, increasing heavy rainfall and strong wind (IPCC 2014) and should be carefully monitored.

Except for the mud deposition in Run 5, we have no data explaining the lower survival rate for I1 and/or I2
compared to O1 and/or O2 in each run. Although low salinities (<20 approximately) continuing for 1 day or more are reported to cause a decreased survival rate in *Atrina* sp. (Kurihara et al. 2017), such low salinities were not found during each run (Table 4). Although the inner area of the Ariake Sea near the sea bed has in general lower dissolved oxygen concentrations than the outer area (Orita et al. 2015), we have no data regarding whether dissolved oxygen concentration became so low (e.g. approximately <0.2 mg L<sup>-1</sup> over a 1.5 day period; Nagasoe et al. 2020) that the pen shells transplanted to some stations died during some runs. Other possible, but unverifiable, factors causing the difference in survival rate include hydrogen sulphide, viruses, parasites, food deficiency, and highly turbid water (Koga & Aramaki 2013; Ito 2017).

We found that transplanted *Atrina* sp. survived better in the outer area of the Ariake Sea than the inner area. In previous transplant experiments in the Ariake Sea, survival rates of *Atrina* spp. were compared between stations exclusively in the inner area of this sea (Ishida et al. 1998; Nasu et al. 2003; Yoshida et al. 2007; Ohashi & Tsukuda 2010; Aramaki & Tsukuda 2014; Matoba et al. 2016; Fukumoto et al. 2017b; Iwanaga 2017). Further studies are necessary to examine which of our finding is applicable to various seasons, years, and age classes. Our transplant experiments were limited to several months (June to October in general), two years (2017 and 2018), and one age class (about 1 year old). Although a decrease in survival rate of pen shells transplanted in the Ariake Sea has often been found for these months (Nasu et al. 2003, 2004; Yoshida et al. 2007; Aramaki & Tsukuda 2014; Matoba et al. 2016), it was also found in other months such as April to June (Yoshida et al. 2007; Aramaki & Tsukuda 2014), March (Matoba et al. 2016), and November to December (Matoba et al. 2016; Iwanaga 2017). Survival rates of transplanted *Atrina* sp. are likely to vary among years, as suggested from the present and previous studies. That is, survival rates per 30 days varied much among 1 year-old *Atrina* sp. that were transplanted to the station 12 and neighboring sites and were protected from predators: 0.844 in 2003 (Nasu et al. 2004), 0.156 in 2004 (Yoshida et al. 2007), and 0.666 in 2015 (Matoba et al. 2016), 0.742 in 2017, and 0.923 in 2018 (the protected cages in the present study). Pen shells of 1 year old and other ages can respond differently to environmental stresses, as is reported for other bivalves (Gosling 2015); such age dependence of *Atrina* sp. is demonstrated by the response to hypoxic stress at least (Nagasoe et al. 2020). Therefore, transplant experiments in future need to be scaled up to all seasons and age classes to examine whether *Atrina* sp. survives better in the outer area than in the inner area of the Ariake Sea.

**Higher survival rate of pen shells in cages with smaller mesh openings**

Studies on how to protect transplanted *Atrina* spp. have largely focused on prevention of predation by the ray *A. narutobiei* (Kawahara et al. 2004b; Fukumoto et al. 2017b). This is presumably because the ray is considered to consume a large amount of bivalves including *Atrina* spp. (Yamaguchi et al. 2005). For instance, in 2009 the population of the ray in the Ariake Sea was estimated to consume >2,000 metric tons of bivalves, which amounts to nearly half of the fishery catch of bivalves therein (Comprehensive Survey Evaluation Committee 2017). Preventive measures against the ray’s predation include rods stuck in the sea bed, which are recommended to be arranged with interstices <15 cm (Kumamoto Prefectural Fisheries Research Center 2017). Another preventive measure is a net with a large mesh opening (5 cm×5 cm by Kurihara et al. 2004b and Fukumoto et al. 2017b).

Protection of transplanted *Atrina* spp. should not, however, be limited to prevention of the predation by the ray. This is indicated from Runs 4 and 5. In these runs, all the cages containing the pen shells had small mesh openings (≤30 mm for Run 4 and ≤115 mm for Run 5), so that the ray should have been unable to eat pen shells in any of the cages. Nevertheless, of these cages, those with smaller mesh openings showed higher survival rates of pen shells, and thus factors other than predation by the ray are also likely to greatly influence their survival rates.

For Run 5 the factors influencing survival rates are likely to be predation by animals smaller than the ray. The reason is that the small predatory animals were often recorded by the time-lapse camera and were found inside the exposed cages with a 115 mm mesh opening (personal communication with divers; Kurihara personal observation). The predatory animals recorded include *R. venosa*, Octopodidae spp., and *Charybdis* spp., which were observed to eat *Atrina* sp. in experimental tanks (Kurihara 2020a, b, c). Although the time-lapse camera also recorded starfish (Asteroidea), which are reported to often prey on bivalves (Gosling 2015), starfish are unlikely to eat the transplanted pen shell. This is because the starfish recorded was presumably *Astropecten scoparius* Müller & Troschel, 1842 smaller than about 10 cm in arm length (Kurihara personal observation). *Astropecten scoparius* of this size is unlikely to prey on the transplanted pen shell larger than about 10 cm in shell length (Table 1), considering that it is reported to eat mostly animals smaller than a few centimeters (Doi 1975; Mukai 1981).

Although various cages used in Run 4 apparently have similar ability to protect from predators since they are made of meshes of similar opening sizes (10, 20 and 30 mm) compared to the cages in Run 5 (15 and 115 mm), we still consider small predatory animals to cause the lower *Atrina* survival rates for larger mesh openings in Run 4. That is, even such a small variation in mesh opening can lead to a large variation in probability of success for soft-bodied, predatory animals to attack the pen shells in the cages. Such animals include octopus, which was recorded by the time lapse camera in Run 5 and was observed to eat a pen shell in an experimental tank (Kurihara 2020a). A
field experiment simulating an octopus pot fishery (Kim et al. 2013) suggests that the success probability for an octopus to attack a prey in a cage greatly varies owing to slight differences in the mesh opening sizes of the cage. For example, Kim et al. indicate that *Octopus minor* (Sasaki, 1920) of 7 cm mantle length (about 40 cm in total length; estimated from its photograph in Okutani 2000) trapped in different net pots with slightly different mesh openings (16 mm and 26 mm) can escape from the pots with markedly different probabilities (<5% and >80%, respectively).

Except for predation by small animals, we have no explanation at present for the lower survival rates for the larger mesh opening sizes. Physical and chemical environmental factors inside cages with larger mesh openings would improve in general (e.g. higher oxidation of sediment) owing to the greater water exchange rate (Miller & Gaylord 2007). Therefore, such environmental factors cannot explain the lower survival rates for larger mesh openings. Although pen shells might have dispersed from the coarse mesh of the lids of the exposed cages in Run 5 (the final size of mesh opening: 115 mm) owing to the strong tidal currents, the possibility of such dispersion is very low. This is because the mesh opening size of lid of the exposed cage had been 15 mm for about two weeks at the beginning of the experiment and thus pen shells had enough time to fix themselves in the sea bed. It should be noted that smaller mesh openings would lead to lower predation risk but also greater fouling of the mesh, which leads to a deterioration of the environment conditions inside a cage (Miller & Gaylord 2007). How to alleviate this problem (e.g. regular cleaning of the mesh) should be studied in future.

Pen shells had better be protected from small predatory animals by cages such that mesh opening is small as suggested above; and also that the material is robust and the side face is embedded in the sea bed. The importance of robustness of material is suggested from the highest survival rates of pen shells in the robust cages (Fig. 1d). Other equipment (Fig. 1a, b, c) was made of soft polyethylene net on the upper face, which the gastropod *R. venosa* sometimes adhered to and bent by its own weight, thereby touching and attacking the pen shells therein (Kurihara personal observation). The soft net was also sometimes cut, and predatory animals (Table 5) accessed the pen shells. The importance of embedding the side face of cages is suggested from the lowest survival rates of pen shells in the flat-wide cages (Fig. 1a). For only the flat-wide cage the side face was not embedded, so that predatory animals were likely to invade the cage by digging through an interstice between the side face and sea bed (Kurihara personal observation).

Concluding remarks

The outer area of the Ariake Sea is a promising area to transplant the pen shell, *Atrina* sp., in terms of enhancing the survival rate. Transplanted pen shells should be protected from predators including not only the longheaded eagle ray *A. narutobiei* but also other smaller animals.

Acknowledgements

Seed production of the pen shell indispensable for the present study was assisted by Nagasaki Prefectural Institute of Fisheries, Saga Prefectural Ariake Fisheries Research and Development Center, Fukuoka Fisheries and Marine Technology Research Center Ariakekai Laboratory, and Kumamoto Prefectural Fisheries Research Center. The present fieldwork was supported by Japan Justice Co., Ltd., Nishimura Syokai Co., Ltd., Saga Ariake Fishery Cooperative Oura Branch, and Shimabara Fishery Cooperative. The earlier version of this paper was much improved by the comments from two anonymous reviewers. The present study was financially supported by contract work with Ministry of the Environment, Japan. We heartily thank all for their assistance.

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