The effect of tube trap structure on sampling efficacy and accuracy for golden mussel, Limnoperna fortunei

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Abstract: The study was conducted to identify the effect of different types of tube traps on the sampling efficacy and accuracy for invasive golden mussels, Limnoperna fortunei (Dunker, 1857), at four sites in Japan. The traps consisted of PVC tubes (diameter: 5.5 cm; length: 20 cm) inside which a PVC plate was fixed horizontally as settlement substrate, and we examined two trap characteristics: mesh covering at the tube ends (present or not) and substrate texture (even or uneven). Mesh covering had a negative effect ($P<0.001$) and uneven substrate had a marginally positive effect ($P=0.08$) on mussel settlement density. A positive relationship was found between larval and settlement density in traps without mesh. These results imply that the most effective type of tube trap had no mesh covering and used uneven substrate. The settlement density of the mussels was also examined on several surfaces of the tube trap and the rope used to suspend the trap. The mussel density on the rope was higher than that on the PVC plates and tubes. The best correlation between settlement density and larval density occurred on the outside of the PVC tubes.

Key words: golden mussel, Limnoperna fortunei, sampling method

Introduction

Recently, it was reported that freshwater biofoulers, including several bivalves (especially zebra mussels, Dreissena polymorpha, golden mussels, Limnoperna fortunei, and asian clams, Corbicula fluminea), are damaging water utilization facilities around the world, and this risk will further increase in the future (Nakano & Strayer 2014). Therefore, it is necessary to develop survey equipment that can easily detect the quantitative distribution and abundance of these species.

Golden mussel, L. fortunei (Dunker, 1857), is a small freshwater bivalve species native to China and Korea (Miller & McClure 1931, Morton 1973, Tominaga & Kimura 2012). This invasive epifaunal species is harmful to natural and man-made structures, such as water-treatment systems and power stations (Ricciardi 1998), and has spread to many areas in Asia (Hong Kong, Japan, Taiwan) and South America (Argentina, Brazil, Paraguay, Uruguay, Bolivia) (Ricciardi 1998, Boltovskoy et al. 2006, Oliveira et al. 2006, Darrigran 2010). In Japan, L. fortunei was first recorded in 1990, in the Ibi River (Kimura 1994), but the organism has spread to the rivers and lakes of central Japan, and is now distributed in 11 of the nation’s 47 prefectures (Ito 2008, National Institute for Environmental Studies 2018).

Recently, golden mussels have been found in various components of water-supply systems in Japan, such as water purification plants (Magara et al. 2001), reservoirs and canals (Katayama et al. 2005, Yoshida 2006), and pipelines (Ito 2008, 2010). Measures to contain and eradicate golden mussels are still insufficient, in part due to uncertainty regarding their distribution and abundance. In Japan, agriculture accounts for over 60% of water usage (Yoshimura et al. 2005). The majority of irrigation facilities are managed by land improvement districts, which are composed of the users of agricultural water (farmers) (Sato 2001), and most of these facilities do not yet monitor L. fortunei. To improve this situation, a simple, broadly practicable monitoring method must be developed.

To prevent the expansion of this invasive species and reduce the damage it causes, early countermeasures based on quantitative abundance data are essential. However, quantitative sampling in water facilities by using conven-
tional methods (such as quadrat sampling) is not effective because golden mussels sometimes attach to non-planar substrates (e.g. corners, water gates, etc.) and are distributed in patches. In addition, it can be difficult to directly observe these facilities because water cannot be withdrawn easily from irrigation ponds and reservoirs while the irrigation facilities are in use. Molecular biological techniques that have been advanced in recent years (such as those using environmental DNA), are thought to be effective for detecting the presence or absence of invasive aquatic organisms, although these methods are not yet able to provide quantitative data (Pie et al. 2017; Xia et al., 2017). In addition, the density of larvae that can be used as an indicator of the abundance of the mussel fluctuates drastically during the reproductive season (e.g. Nakano et al. 2017), so a high frequency of investigation is required. In order to counteract biofouling at water utilization facilities, however, a simple method which requires a low frequency of investigation is required.

When benthic organisms cannot be sampled effectively by conventional methods, estimates of abundance obtained from the colonization of artificial substrates can be an effective method of collecting data. The use of artificial substrates standardizes sampling, and reduces variability and sample-processing time (Gibbons et al. 1993). So far, attempts have been made to clarify the abundance of golden mussels in the field by investigating various artificial substrates at a low frequency, including three-dimensional asbestos structures (Morton 1977), PVC-plate adaptations (Boltovsksoy & Cataldo 1999), plastic containers (Nakano et al. 2010), fiber cement plates (Darrigran et al. 2007), ceramic substrates (Bergonci et al. 2009), and nylon nets (Oliveira et al. 2011). However, few studies have exactly investigated the extent to which the data obtained from artificial substrates reflect larval density.

We think that the traps used for monitoring golden mussels should have the following two characteristics. First, to avoid overlooking invasions by this organism, the trap should capture sufficient numbers of mussels. Second, the settlement density on the trap should be highly correlated with actual mussel density (larval or adult) in the sampled environment. Most field monitoring efforts assume that these relationships are highly significant, but this assumption has not yet been fully confirmed.

The main aim of this study was to clarify the effect of trap characteristics on sampling efficacy and accuracy for settlement density of golden mussels. This study examines: (1) the effects of the trap characteristics ‘net covering’ and ‘uneven substrate’ on the settlement density of L. fortunei; and (2) the relationships between larval density in water and settlement density in traps. In light of the results, we discuss effective trap design for early detection and monitoring of this organism.
Materials and Methods

Site description

The field experiments were conducted at three sites in the Kanto region, namely the Sasagawa sediment basin (SS; 35°50′13″N, 140°41′12″E), the Minamishiio Reservoir (MR; 36°4′15″N, 140°4′15″E), and the Kasumigaura Pumping Station (KP; 36°3′52″N, 140°19′18″E), and at one site in the Chubu-Kinki region, the Nagara River (NR; 35°4′45″N, 136°41′40″E). SS is a small pond next to the Kurobe River, and is connected to the sluice gate of the Toso irrigation canal in northern Chiba prefecture. Golden mussels have been found in the Toso irrigation canal since 2008. MR and KP are reservoirs of the Kasumigaura canal, which is a large pipeline system that is connected to Lake Kasumigaura, Ibaraki Prefecture. Golden mussels have been found in Lake Kasumigaura since 2005 (Sunoh 2006). NR is upstream of the Nagara River mouth barrage in Mie Prefecture. Golden mussels have been found in Lake Kasumigaura since 2005 (Sunoh 2006). NR is upstream of the Nagara River mouth barrage in Mie Prefecture. Golden mussels have been found in the lower reaches of the Nagara River since 1993 (Nakai et al. 1994). The water in the SS, MR, and KP water bodies is calm, with an absence of currents, and the flow of water in the Nagara River is controlled by the barrage at the river’s mouth.

Experimental design

We used tube traps to test the effect of trap characteristics on the sampling efficacy for golden mussels (Fig. 2). The traps consisted of a PVC tube (diameter: 5.5 cm; length: 20 cm) fitted with a PVC plate (5 cm × 20 cm × 2 mm) oriented horizontally to act as a settlement substrate. We examined two characteristics of the trap: the use of mesh over the openings (mesh compared to no mesh) and substrate texture (even compared to uneven). For the mesh-containing traps, both ends were covered with mesh (mesh openings, 4 mm). An uneven substrate was created by coiling 1 m of rope (diameter, 4 mm) around the internal plate (Fig. 2D). To counter the effect of silt deposition on the substrate, mussels were collected from the underside of the plate only. At each installation, two traps of the same type were installed (one each at depths of 1.0 and 1.5 m), and traps were suspended on a rope fixed to a buoy (Fig. 2).

Previous studies suggest that water depth affects the settlement density of golden mussels (Morton 1977, Nakano et al. 2010). Because the maximal water depth at all of our sites was 3 to 5 m; we conducted the field experiment only in the shallower areas and ignored the effect of water depth. There was no difference in mussel settlement density between upper and lower traps (see Results).

The two levels of the two treatments were combined factorially to create four types of trap: mesh-covered trap with uneven substrate (uneven plate with mesh), open trap with uneven substrate (uneven plate without mesh), mesh-covered trap with even substrate (even plate with mesh), and open trap with even substrate (even plate without mesh). Ropes with two traps were deployed in a random-
ized block design, with each block consisting of one set of the four types of trap. Thus, this experiment incorporated a 2-factor, randomized block design with ‘mesh covering’ and ‘uneven substrate’ as main effects. Each of the four research sites contained three blocks, using a total of 48 ropes (96 traps). At the Kanto region sites (SS, KP, MR), ropes were set 2 to 4 m apart from each other within each block, and each block was separated from other blocks by 5 to 130 m. Because the space for installing traps at the NR site was limited, the ropes and blocks were separated by 1 m.

In Japanese freshwater (the Uji River, Kyoto Prefecture), *L. fortunei* reproduce from June to September, and newly settled juveniles were found from July to September (Iwasaki & Uryu, 1995). The experiment began with the setting of the ropes in July 2009, and ended in December 2009 with the removal and processing of traps. After removal, traps were immediately immersed in 80% ethanol. In the laboratory, all individual *L. fortunei* were removed from the substrate using a nylon brush. The removed mussels were sieved (mesh opening, 0.25 mm), and the retained organisms were preserved in 80% ethanol. The *L. fortunei* retained on the sieve were counted using a binocular microscope. Before data analysis, the counts from the two traps (at 1 m and 1.5 m depth) attached to the same rope were pooled. During the experiment, one rope (uneven plate with mesh’ trap) was lost at the NR site, causing one missing data replicate from an otherwise complete set.

Golden mussels attached not only to the PVC plate, but also to other parts of the tube traps. To examine the settlement patterns of mussels on the traps, we collected the mussels from all surfaces of the traps with open tubes and even substrate. The trap surfaces were divided into five different parts: (1) ropes (diameter: 6 mm; length: 20 cm; five sections of rope per trap); (2) PVC plate (upper side=lower side); (3) tube internal surface (end sleeve); (4) tube internal surface (inner chamber); and (5) tube external surface (Fig. 3). The counts from the two traps on the same rope (at 1 m and 1.5 m depth) were pooled for each location.

During the experiment, the traps were checked at approximately the same time each month. The screen net was obstructed by various sessile organisms, such as magnific cent bryozoa, *Pectinatella magnifica*, especially in summer. During monthly visits, all sessile organisms except *L. fortunei* were removed from traps as much as possible. During the monthly visits, we measured pH, dissolved oxygen concentration, and electrical conductivity. Throughout the experimental period, we recorded water temperature at 1 m depth every hour at a single location at each site using a data logger (Tiny Tag Plus, Gemini Data Loggers Ltd, Chichester, UK).

To obtain a measure of the variation in the density of free-swimming *L. fortunei* larvae, samples were obtained monthly by means of a plankton net (diameter: 20 cm; mesh size: 72 μm, Rigosha & Co., Ltd. Saitama, Japan) at each sampling site. The plankton net was hauled vertically several times (depending on the depth) from the bottom to the surface (total volume of water filtered was approximately 1 m³). The samples were preserved in 80% ethanol. Shelled-stage larvae (straight-hinged veliger to pediveliger, Cataldo et al. 2005) were counted under a binocular microscope in the laboratory.

**Statistical analysis**

To examine the effects of the trap characteristics ‘net covering’ and ‘uneven substrate’ on the settlement density of *L. fortunei*, we used a generalized linear model procedure with Poisson (log link) error structure. When over-dispersion in the count data (dispersion parameter > 1; variance > mean) was present, we fit the data with a negative binomial distribution or quasi-Poisson (log link) error structure (Zuur et al. 2009). The explanatory variables were the two trap characteristics and the interaction between them, block, and sampling site. Factors that are not considered in the effect size (i.e., block and sampling site) are recommended to be used as random effects in the generalized linear mixed model (GLMMs) (Crawley 2005). However, we could not successfully converge the model using GLMMs. In addition, adding random effects with too few levels (fewer than four level) leads to imprecise estimates of the standard deviation in GLMMs (Bolker et al. 2009). Therefore, we regarded both block and sampling site as fixed effects, and assumed that there was no interaction between these factors and trap characteristics. The area of the substrate (200 cm² for plain PVC plates, 286 cm² for PVC plates coiled with rope) was used as an offset term. To test the significance of explanatory variables, we used the Chi-square test (Poisson and negative binomial error) or F-test (quasi-Poisson error) in a type II analysis of deviance (Faraway 2006). The generalized linear model analyses were performed both with all the data from all four sites (*n*=47) and individually for each site (*n*=12, except NR [*n*=11]).

To examine the settlement pattern in the tube traps, we again used generalized linear models with Poisson (log
link) or negative binomial error structures. The explanatory variables included sampling position, site, and block. In addition, the substrate area was used as an offset term. In the calculation of the surface area of the rope, the fine surface structure was ignored. The Tukey multiple comparison test was used to detect differences in the density of *L. fortunei* recovered from the different parts of the trap at each site.

To evaluate the performance of trap type, the relationship between larval density in water and settlement density in traps was analyzed for each type of trap. We took only one larval-density measurement at each site, and ignored the larval-density variation among blocks. The analysis was performed by using the method for more than one value of *y* (settlement density) for each value of *x* (larval density) (Sokal & Rohlf 1995). All abundance data were log(*x*+0.5)-transformed in this analysis (Yamamura 1999). The relationship between settlement density at different positions of the trap and larval density was also examined.

All statistical analyses were performed with R 3.4.0 software (R Core Team 2017). The GLM analyses with Poisson and quasi-Poisson error were performed by using the `glm` function. The negative binomial regression analysis was conducted by using the `glm.nb` function in the MASS library. The `glht` function (multcomp library) was used for the multiple comparison of settlement patterns at different trap positions. For the analyses, a *P*-value less than 0.05 was considered statistically significant.

### Results

The settlement density of mussels at the sampling sites ranged from 0.01 ± 0.01 cm⁻² (mean ± SD) at NR, to 0.37 ± 0.41 cm⁻² at SS (Table 1). There was no statistical difference of mussel settlement density between upper and lower traps (exact Wilcoxon rank sum test, *v* = 319.5, *P* = 0.330). The environmental characteristics of these sites are listed in Table 2. The pH of water samples from the SS site was significantly lower than that at the other sites (two-way ANOVA with Dunnett’s multiple comparison test, *P* < 0.05)

Trap structure affected the settlement density of *L. fortunei* (Figs. 4 and 5, and Table 3). Overall (Table 3, ‘All sites’), the mesh covering had a negative effect (*P* < 0.001) and the uneven substrate had a marginally positive effect (*P* = 0.084) on mussel density. In addition, the interaction between the two treatments was significant (*P* < 0.05). The main effects differed slightly between the sites (Fig. 5, Table 3). At the sites with high mussel density (SS and MR), the main effects of the treatments were significant (*P* < 0.05). In contrast, most of the treatment main effects were not significant at the low-density sites (KR and NR). The block effect was significant (*P* < 0.05) at all sites except NR, where the distance between blocks was only 1 m.

During the experiment, a wide variety of sessile organ-

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**Table 1.** Settlement and larval density of *Limnoperina fortunei* at each sampling site.

| Site          | Settlement density (number cm⁻²) | Larval density (number m⁻²)* |
|---------------|----------------------------------|------------------------------|
| Sasagawa (SS) | 0.37 ± 0.41                      | 18,800 ± 13,800              |
| Kasumigaura (KR) | 0.03 ± 0.04                  | 0.33 ± 0.58                  |
| Minamishio (MR) | 0.17 ± 0.18                  | 68.00 ± 116                  |
| Nagara River (NR) | 0.01 ± 0.01                  | 1.33 ± 1.53                  |

Data are given as means±1 SD. *Average value of the monthly sampling data (July to September 2009)

**Table 2.** Environmental characteristics of the study sites measured from July to December 2009.

| Site          | Water Temperature (°C) | Dissolved O₂ (mg L⁻¹)* | pH          | Conductivity (mS cm⁻¹)* |
|---------------|------------------------|------------------------|-------------|------------------------|
| Sasagawa (SS) | 20.8 ± 5.6             | 6.70 ± 2.81            | 7.22 ± 0.12 | 22.9 ± 3.2             |
| Kasumigaura (KR) | 21.1 ± 5.8             | 9.88 ± 2.19            | 8.41 ± 0.63 | 21.0 ± 6.3             |
| Minamishio (MR) | 21.5 ± 5.7             | 9.52 ± 3.16            | 8.18 ± 0.89 | 21.1 ± 6.1             |
| Nagara River (NR) | 19.6 ± 4.8             | 2.87 ± 0.63            | 8.17 ± 0.67 | 9.6 ± 1.2              |

Data are presented as means±1 SD. *Measured by using a data logger hourly at a depth of 1 m at a single location per site. †Measured monthly during monitoring visits.

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**Fig. 4.** Box plot distributions of the settlement density of *Limnoperina fortunei* in each of the four types of trap for all data from the four sites combined. Data are shown as box plot diagrams showing medians (line in the boxes), 25% and 75% percentiles (boxes). Upper and lower vertical lines on the columns represent maximum and minimum values, respectively.
isms attached to the tube traps (Fig. 6). Many species of bryozoa, freshwater sponges, and algae were found on the surfaces of the tubes, nets, and plates. Sometimes, the tubes with the mesh covering were obstructed by sessile organisms. At SS and NR, the traps were covered with magnificent bryozoan during the summer (Fig. 6). Fish (e.g., dusky tripletooth goby, *Tridentiger obscurus*), shrimp (*Palaemon* sp.), and clams (*Corbicula fluminea*) were also found in the traps (including those with mesh-covered openings), but were removed by hand during the monthly inspections (along with sessile organisms other than *L. for-
tunei*).

Individuals of *L. fortunei* were found attached not only to the PVC plate installed in the tubes, but also to the other parts of the trap (Figs. 7, 8). At the high-density sites (e.g., SS), numerous mussels were found on the rope and internal and external surfaces of the tubes (Fig. 8). The overall densities of the mussels varied between the parts of the trap (generalized linear model with negative binomial error structure, \( \chi^2 = 35.686, P < 0.001 \)). The parts of the traps with the highest density of mussels tended to be the rope and the internal surface of the end sleeve (Fig. 8).

The site with the highest settlement density, SS, also had the highest larval density among the four sites (Table 1). The relationship between settlement density and larval density differed with trap type (Fig. 9). A positive relationship between larval density and settlement density was evident for ‘uneven plate without mesh’ traps \( (F_{(1, 2)} = 18.71, P < 0.05) \) and ‘even plate without mesh’ traps \( (F_{(1, 2)} = 17.87, P = 0.052) \), but not for ‘uneven plate with mesh’ traps \( (F_{(1, 2)} = 3.81, P = 0.19) \) or ‘even plate with mesh’ traps \( (F_{(1, 2)} = 1.23, P = 0.38) \).

The relationship between settlement density and larval density also differed with the position of the traps (Fig. 10). A positive relationship between larval density and settlement density emerged for rope \( (F_{(1, 2)} = 52.96, P < 0.05) \), tube external surface \( (F_{(1, 2)} = 327.95, P < 0.01) \), end sleeve \( (F_{(1, 2)} = 83.65, P < 0.05) \), and PVC plate \( (F_{(1, 2)} = 26.12, P < 0.05) \), but not inner chamber \( (F_{(1, 2)} = 6.16, P = 0.131) \).

**Discussion**

As mentioned earlier, we think that the traps used for monitoring golden mussels (*L. fortunei*) should have the two characteristics of high collection efficiency and accurate representation of mussel (larval) density. In our results, the mesh covering had a negative effect, and the uneven substrate had a slightly positive effect on mussel settlement density (Table 3). A positive relationship was found between larval and settlement density in traps with-
out mesh covering (Fig. 9). These results imply that the most effective type of tube trap was the 'uneven plate without mesh' trap in our experiment. The significant block effect (Table 3) means that the density varies markedly depending on the location of the trap. To prevent overlooking invading organisms, it would be desirable to have as many traps as possible.

At the high-density sites, the density of mussels in the

Table 3. The effect of trap structure on the recruitment density of *Limnoperna fortunei*, at all sites overall and at each of the four study sites.

| Independent variables | Estimated value | SE  | F  | LR $\chi^2$ | df | $P$ |
|-----------------------|-----------------|-----|----|-------------|----|-----|
| **All sites (model applied, negative binomial with parameter $k=3.55$)** | | | | | | |
| Mesh                  | -0.591          | 0.272 | —  | 28.404      | 1  | <0.001 |
| Uneven                | 0.717           | 0.250 | —  | 2.991       | 1  | 0.084  |
| Site                  | —               | —    | —  | 162.433     | 3  | <0.001 |
| Mesh $\times$ Uneven  | -0.869          | 0.377 | —  | 4.974       | 1  | <0.05  |
| Site $\times$ Block   | —               | —    | —  | 49.669      | 8  | <0.001 |
| **Sasagawa sediment basin (model applied, negative binomial with parameter $k=5.12$)** | | | | | | |
| Mesh                  | -1.4147         | 0.394 | —  | 32.337      | 1  | <0.001 |
| Uneven                | 0.7843          | 0.370 | —  | 4.013       | 1  | <0.05  |
| Mesh $\times$ Uneven  | -0.4545         | 0.546 | —  | 0.671       | 1  | 0.413  |
| Block                 | —               | —    | —  | 7.698       | 2  | <0.05  |
| **Minamishiio Reservoir (model applied, quasi-Poisson with dispersion parameter=2.97)** | | | | | | |
| Mesh                  | -1.116          | 0.318 | 46.173 | —         | 1  | <0.001 |
| Uneven                | 0.476           | 0.189 | 9.147 | —         | 1  | <0.05  |
| Mesh $\times$ Uneven  | 0.024           | 0.380 | 0.004 | —         | 1  | 0.951  |
| Block                 | —               | —    | 47.154 | —         | 2  | <0.001 |
| **Kasumigaura Pumping Station (model applied, Poisson)** | | | | | | |
| Mesh                  | 2.674           | 0.731 | —  | 0.392      | 1  | 0.532  |
| Uneven                | 2.799           | 0.722 | —  | 2.160      | 1  | 0.142  |
| Mesh $\times$ Uneven  | -3.885          | 0.792 | —  | 46.597     | 1  | <0.001 |
| Block                 | —               | —    | —  | 45.584     | 2  | <0.001 |
| **Nagara River (model applied, Poisson)** | | | | | | |
| Mesh                  | -2.078          | 0.757 | —  | 22.935     | 1  | <0.001 |
| Uneven                | -0.164          | 0.361 | —  | 0.311      | 1  | 0.577  |
| Mesh $\times$ Uneven  | -0.357          | 1.286 | —  | 0.079      | 1  | 0.778  |
| Block                 | —               | —    | —  | 0.047      | 2  | 0.977  |

Data shown are the results of generalized linear model analysis. The estimates for "Mesh" indicate relative height of "mesh covered trap" compared with "open trap", and estimates for "Uneven" show the relative height of "uneven substratum" compared to "even substratum" (see Materials and Methods).

Fig. 6. Traps with sessile organisms attached. Mesh covering blocked with sessile organisms (A). Trap with large colonies of magnificent bryozoan, *Pectinatella magnifica* (B).
Fig. 7. *Limnoperna fortunei* attached to a trap at a high-density site (Sasagawa sediment basin). Complete trap (A). Opening of the tube (B). Uneven plate (C).

Fig. 8. Box plot distributions of the settlement density of *Limnoperna fortunei* at different locations on the trap ('even plate without mesh' trap). Data shown as box plot diagrams showing medians (line in the boxes), 25% and 75% percentiles (boxes). Upper and lower vertical lines on the columns represent maximum and minimum values, respectively. Abbreviations: SS, Sasagawa sediment basin; KP, Kasumigaura pumping station; MR, Minamishiio Reservoir; NR, Nagara River. Distributions labeled with the same letter are not significantly different at $P=0.05$. 
traps covered with mesh was lower than in the open traps (Fig. 5). This trend contrasted with the results of previous studies, in which mesh-covering increased the mussel settlement densities (e.g., Sylvester et al. 2007, Nakano et al. 2010). Previous studies concluded that the greater mussel density in the mesh-covered treatment was mainly a result of the exclusion of predators (Sylvester et al. 2007, Nakano et al. 2010). This discrepancy might reflect the large number of other sessile organisms that attached to the mesh covers in our study (Fig. 6). Attachment of organisms to the mesh may prevent the mussel larvae from passing through the mesh, and may reduce the survival rate in the trap (due to a decrease in dissolved oxygen content, etc.). In contrast to our research, Nakano et al. (2010) found no evidence for the obstruction of \( L. \) fortunei settlement by other sessile organisms on the mesh. Under the conditions of the current experiments (shallow water, slow water current, many sessile organisms, etc.), we cannot recommend covering the end caps of the tube traps with mesh. Further research is needed to determine whether monitoring traps should include mesh in general.

To investigate the effect of treatments, we analyzed the mussel densities on the PVC plates inserted into the tubes. Throughout the experiment, however, golden mussels adhered more frequently to the ropes than the plates (Figs. 5 and 8). Rope is likely superior to PVC as a settlement substrate because the rope surface is not uniform. If collection efficiency is the key criterion, rope itself may be the preferred settlement substrate. Nakano et al. (2012) examined settlement of \( L. \) fortunei using unraveled ropes, and found that the density of larvae and post-larvae have the same tendency.

Settlement density and larval density were significantly correlated on most surfaces of open-tube traps, with the highest correlation on the outside of the tube (Fig 10, \( R^2 = 0.9118 \)). This result suggests that the settlement density of golden mussels on the surfaces of PVC tubes is an effective indicator of variations in larval density. The method of submerging PVC pipes was also used for monitoring zebra mussels, \( D. \) polymorpha (Kraft 1993). Such a method may be superior to quantitative monitoring of golden mussel larvae.

In light of our current results, we offer the following comments regarding a practical method for using similar traps for monitoring golden mussel. Interest in investigating the organism tends to be low in un-invaded areas. In this situation, a simple, inexpensive sampling device, with a high collection efficiency is required. Because detection

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**Fig. 9.** The relationship between larval density (sum of larvae sampled [number m\(^{-3}\)] from July through September) and settlement density of \( L. \) fortunei in the four types of trap. The regression line of the solid line represents a statistically significant relationship (\( P < 0.05 \)), and the regression line of the dotted line represents a statistically weaker relationship (\( 0.05 < P < 0.1 \)). The relationships between larval and settlement density in the traps with mesh were not significant (\( P > 0.1 \)).
of an invasion is more important than the accuracy of the density estimation under these conditions, we think that a simple survey using rope only would be beneficial, especially when it contains multiple knots, given that the mussels tend to concentrate in these areas (Fig. 7A, Ito K., unpublished data). When it is important to evaluate the larval density, we recommend using PVC tubes without mesh covering as traps. In addition, because the mussel density on PVC tubes is relatively low, materials with high adhesion efficiency (e.g., rope) should be checked simultaneously. Finally, as many traps as possible should be installed, to counteract location-dependent variations in density (Table 1, block effect).

Various methods for early detection of golden mussels, such as molecular analysis (Pie et al., 2006, Boeger et al., 2007, Endo et al., 2009, Pie et al., 2017) have recently been developed. Although these methods are very useful (Darrigran et al. 2009), it may be preferable to use simple traps in situations where there are few available funds for detecting the mussels. In the current study, we have clarified some of the key characteristics that influence the efficacy of simple traps composed of ropes, PVC plates, and tubes. These findings will be useful for investigating golden mussels more effectively.

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