Numerical study on evaluation of environmental DNA approach for estimating fish abundance and distribution in semi-enclosed bay

Running title: eDNA approach for estimating fish abundance

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Abstract

Although environmental DNA (eDNA) of aquatic species has been widely explored, the quantitative assessments of fish remain a challenge. eDNA approach proposed by Fukaya et al. (2021) gave a reliable abundance (the total fish population in the study area) estimates of coastal fish but was not as useful for assessing spatial distribution because of fewer eDNA samples relative to the study area. Hence, we evaluated the ability of the eDNA approach to estimate the abundance and distribution of fish in a semi-enclosed bay based on a numerical study. The evaluation was conducted as a case study on the ability of the eDNA approach according to the number of eDNA samples. Our study revealed that the eDNA approach can reliably estimate fish abundance regardless of the number of eDNA samples, if outliers of the fish density estimates are eliminated. However, when estimating spatial distribution, significant estimates were obtained only under those conditions wherein the eDNA concentration was identified in more than 70% of the study area. Therefore, it is necessary to explore other methodologies for broadly estimating eDNA concentrations with fewer samples. We have confirmed that the eDNA approach can reflect fish abundance but has limitations in estimating fish distribution. From above results, we expect our results to provide researchers with more insights into estimating the abundance and spatial distribution of fish using eDNA.

Keywords: environmental DNA, fish school, case study, number of eDNA samples, tracer model, Jinhae bay

1 Introduction

It is critical to estimate the abundance and distribution of fish for the management and sustainable use of fishery resources. In recent decades, overfishing has become a global challenge as fishing has increased due to population growth and development of civilization (Galbraith et al., 2017; Munro & Bell, 1997). Since the early 1970s, the proportion of sustainably harvested stocks has been gradually decreasing, with a recent estimate of it being only 67% (Sofia, 2018). Quantitative assessments (e.g., estimating the abundance and distribution) of fish should precede for the effective management of overfishing, which can cause ecological imbalances as well as habitat changes in the coastal system (Bach et al., 2022; Pacoureau et al., 2021).

There are conventional methods to estimate fish abundance (e.g., gill-netting, bottom-trawl, mark-recapture, and echo-sounder), and these are classified as fishery-dependent or fishery-independent (Rourke et al., 2022). Fishery-dependent methods are used to statistically estimate fish abundance based on fishery logs (e.g., vessel logbooks). While these are efficient because they are less costly in terms of financial and human resources, they involve many biases, including gear selectivity and variable fishing efforts (Dennis et al., 2015). The variable fishing efforts affect the quantitative evaluation of fishery resources such as the catch per unit effort, and the abundance of target species may be under estimated due to gear selectivity, including the shape or features of gears (Bonar et al., 2009). Fishery-independent methods (e.g., mark-recapture and echo-sounder) are not affected by the gear selectivity because of the use of similar gears (Dennis et al., 2015). Furthermore, since these methods are mainly used for scientific sampling, they provide reliable data for quantitative assessment (Rourke et al., 2022). However, these methods often require expensive equipment and are not as useful for broad scale. Mark-recapture is associated with a high-cost process, namely the repetition of capture-count-mark-release, in terms of human and time resources. Moreover, it does not consider migration, mortality, and recruitment because of the supposition of a closed population, which remains unchanged during the investigation (Seber, 1986). An echo-sounder requires the target strength parameter of the target species. Since the target strength changes depending on the characteristics (e.g., body size and shape) of the target fish, it needs to be estimated individually (Vaughan & Recksiek, 1979). Conventional methods may have limitations such as high-cost, small-scale, biases, habitat disturbance, and mortality. In particular, these methods have an undetected probability for rare species such as endangered and/or protected species.

The environmental DNA (eDNA) approach, which is an emerging method for the investigation of aquatic organisms, is cost-effective, noninvasive, and has been proposed as an alternative to the conventional methods

(Deiner et al., 2017; Hansen et al., 2018). The eDNA methodology is less affected by investigational circumstances (e.g., accessibility and uneven distribution) and could reduce the costs related to data collection (Laramie et al., 2015). Furthermore, it has been evaluated as a way to minimize habitat disturbance because it requires only water samples for analysis (Lacoursière-Roussel, Côté, et al., 2016). In recent years, eDNA approach has shown the ability to reliably quantify aquatic organisms. Specifically, studies have been conducted pertaining to the release and/or degradation rate of eDNA (Klymus et al., 2015; Maruyama et al., 2014; Sassoubre et al., 2016), biodiversity (Andersen et al., 2012; Nakagawa et al., 2018), detection (Baldigo et al., 2017; Eichmiller et al., 2016), abundance (Díaz-Ferguson et al., 2014; Ghosal et al., 2018; Nevers et al., 2018), distribution (Eichmiller et al., 2014; Fukaya et al., 2021; Itakura et al., 2020; Itakura et al., 2019), and comparison with conventional methods (Capo et al., 2019; Lacoursière-Roussel, Rosabal, et al., 2016). Early studies mainly examined the relationship of eDNA with biodiversity and presence of species. Although more recent studies have focused on the abundance and/or distribution of aquatic species, their analyses remain a challenge because of unclear processes such as shedding, degradation, transport, and exogenous input of eDNA in the natural environment. Fukaya et al. (2021) proposed a novel approach, while considering these processes, for estimating the abundance and distribution of jack mackerel (*Trachurus japonicus*) in a coastal bay. They showed that the approach could reliably estimate the abundance of jack mackerel, but the spatial distribution was not as clear. They envisaged that the lower number of eDNA samples relative to the number of grid cells could have led to the unclear spatial distribution. Because of the absence of related studies, it is uncertain whether insufficient eDNA samples caused this disagreement. Most studies pertaining to eDNA have only been conducted since the early 2000s, and those on the estimation of abundance and distribution are very rare.

Herein, we evaluated the eDNA approach to estimate the abundance and distribution of jack mackerel based on a numerical study using a number of eDNA samples relative to the study area as a simulation condition.

2 Materials and methods

2.1 eDNA approach for estimating fish abundance and distribution

The eDNA approach proposed by Fukaya et al. (2021) consists of forward and backward inferences. First, the forward inference was used to calculate the eDNA concentration using the current field, rate parameters, and fish density as inputs. We obtained a design matrix $\boldsymbol{A}$, which is used to calculate the fish density from forward inference. Backward inference was then defined as a process to calculate fish density using matrix $\boldsymbol{A}$ and eDNA

concentrations. We estimated fish density by multiplying $\boldsymbol{A}^{-1}$ with an eDNA concentration vector interpolated to the whole cell with limited known values of eDNA. More details regarding the eDNA approach have been reported by Fukaya et al. (2021).

The tracer model required the current field, rate parameters, and fish density as inputs. The rate parameters were the eDNA shedding rate of fish and degradation rate of eDNA. We used the shedding rate (9.88×10^4 copies individual^{-1} h^{-1}) and degradation rate (0.044 h^{-1}) of jack mackerel, which was our target species introduced in the study by Fukaya et al. (2021) (Jo et al., 2017). We constructed the current field from the Princeton Ocean Model (POM) aimed at Jinhae bay in South Korea and randomized fish density.

2.2 Simulation of current field

The current field simulated in this study was aimed at Jinhae Bay, South Korea (Figure 1). The current field includes hydrodynamic processes (e.g., three-dimensional flow velocity, temperature, salinity, and diffusion coefficient) and those that determine the transport of eDNA in the field. The current field was produced using POM within the bay in approximately one month. Specifically, the model grid was discretized using 74×87 horizontal grid cells with a resolution of 500 m, and the sigma ($\boldsymbol{\sigma}$) coordinate was adopted for the vertical grid with 10 $\boldsymbol{\sigma}$ layers. The total number of grid cells was 64,380 with 24,480 aquatic cells. We then verified the tide level and tidal flow using a time series and tidal ellipse, respectively. The phase lag and amplitude of the tide level showed small errors of 0.5-7.4° and ±1.0 cm, respectively. The calculated tidal current showed good agreement between the calculated and observed values for tidal ellipse and phase. We verified temperature and salinity using the objective functions of determination coefficient (R^2) and skill score (SS). The SS ranges from 0 to 1, and the closer it is to 1, the better the agreement (Willmott, 1981). The R^2 and SS values for water temperature were 0.96 and 0.99, respectively, and those for salinity were 0.74 and 0.93, respectively. More details regarding the current field have been provided by Park et al. (2021).

2.3 Latent fish density for simulation

We randomized the latent fish densities representing the actual fish density for the simulation. We considered the following three cases of latent fish density. Case1: all values randomized to 0-10 individuals m^{-3} (ind. m^{-3}), Case2: high fish density (15 ind. m^{-3}) at a specific point in the surface layer, and Case3: high fish density (15 ind. m^{-3}) at a specific point in the bottom layer. The high fish densities in Case2 and Case3 represent fish schools. We

obtained a total of 15 latent fish densities with 5 densities for each of Case1, Case2, and Case3 (Figure 2).

2.4 Evaluation of the eDNA approach

We calculated the eDNA concentration and design matrix from the tracer model with latent fish density, current field, and rate parameters after stabilization of the model for approximately one month. The eDNA concentrations sampled in the field may under- or over-estimated. Therefore, we considered various known value ratios (KVR; i.e., ratios representing the number of eDNA samples relative to the study area) of eDNA concentrations of top and bottom 1, 3, 5, 7, 10, 20, 30, …, 90%. We first divided the study area and selected known values to prevent the known value distribution from being biased (i.e., top 1% KVR=collection of the top 1% eDNA concentrations selected in each section). We then interpolated the selected eDNA concentrations to whole cells and estimated the fish density by multiplying the eDNA concentration vector by $\boldsymbol{A}^{-1}$. Fish density estimates below zero were set to zero, and outliers were eliminated based on the generalized extreme studentized deviate (GESD) method (Rosner, 1983). The maximum number of outliers in GESD was set at 10% of the total grid cell. Finally, we evaluated the results of estimation of fish abundance and distribution by comparing with those of the latent condition. It is important to assess underestimation and overestimation when evaluating fish abundance. Therefore, we evaluated the estimated fish abundances as a relative ratio (reproducibility) to latent fish abundance using the following equation:

$$Reproducibility = \frac{Estimated\ fish\ abundance}{Latent\ fish\ abundance}$$

If the reproducibility is one, it represents a perfect match, and reproducibility greater than one or less than one represents overestimation or underestimation, respectively. The estimated fish distributions (i.e., spatial distribution of fish densities) were evaluated by visual inspection and correlation coefficient (R) between the estimated and latent fish densities. We then compared the histograms and scatter plots of the estimated fish densities with those of the latent fish densities.

3. Results

3.1 Comparison of estimation accuracies between cases

The evaluated results for fish abundance (reproducibility) and distribution (R) with the top and bottom 1%, 50%, and 90% KVR conditions for all cases are shown in Table 1. The mean reproducibility (averaged for all cases) for the top and bottom 1% KVR was 0.973 and 0.877 with standard deviation of 0.039 and 0.059, respectively. The standard deviation decreased as the KVR increased, with values being 0.004 and 0.003 at the top and bottom 90% KVR, respectively. The reproducibility for the top and bottom 1% KVR for Case3-4 was 1.020 and 1.047, respectively, which was 0.047 and 0.170 higher than the mean values. This may have been overestimated due to insufficient eDNA samples (i.e., low KVR). The difference in reproducibility between the cases was up to 0.120 and 0.229 at the top and bottom 1% KVR, respectively. It is expected that the reproducibility of the estimated fish abundance may vary depending on the fish distribution if the eDNA sample is insufficient. The R values between the latent and estimated fish densities showed a small standard deviation of 0.01-0.03, regardless of the KVR. The R values showed a range of 0.07-0.11, 0.39-0.48, and 0.82-0.86 at the top 1%, 50% and 90% KVR, respectively. There was no significant case-dependent (i.e., fish distribution-dependent) difference between the R values.

3.2 Evaluation of eDNA approach for estimating fish abundance

The evaluation results for fish abundance (reproducibility) with all KVR conditions for Case1-1, Case2-1, and Case3-1 are shown in Table 2. The latent fish abundances for Case1-1, Case2-1, and Case3-1 were 2.95×10^{10}, 3.03×10^{10}, and 2.99×10^{10} individuals, respectively, which were out of proportion for the bay size of 612 km^2. This was caused by the high initialization of latent fish density at 0-10 ind. m^{-3}. It is expected that the estimation accuracy would not differ by changing the fish density scale because our process is linear. The top KVR conditions showing reproducibility closest to the latent fish density were 3% for Case1-1 and Case2-1 and 90% for Case3-1, where the reproducibility was 0.989, 0.974, and 0.973, respectively. In the bottom KVR condition, the 90% KVR showed reproducibility closest to the latent for all cases, with the reproducibility being 0.989, 0.991, and 0.986 for Case1-1, Case2-1, and Case3-1, respectively. The approach showed high reproducibility (>0.800) not only in 90% KVR, but also in low KVR conditions. In the bottom 1% KVR condition, the reproducibility was 0.856, 0.846, and 0.938 for Case1-1, Case2-1, and Case3-1, respectively, and it gradually increased with increasing KVR. The underestimation in both KVR conditions was caused by the elimination of outliers. The reproducibility of all cases before outlier elimination was overestimated to 1.652-1.869 and 1.194-1.453 in the top and bottom 50% KVR, respectively. The eDNA approach could estimate the fish abundance with a reproducibility of over 0.800 (i.e., error under 0.200), regardless of KVR and fish distribution.

3.3 Evaluation of eDNA approach for estimating fish distribution

The estimated fish distribution and R-values between the latent and estimated fish densities are shown in Figure 3, 4 and Table 2, respectively. The R value of the top 1% KVR was 0.10, which was 0.07 higher than the bottom 1% KVR of Case2-1. The difference was whether they were able to estimate the fish school. The top 1% KVR of Case2-1 was partially capable of materializing a fish school, whereas the bottom 1% KVR was not able to do so (Figure 3). The eDNA selection process may make the difference because the eDNA copies shed from a fish school are more likely to be selected in the top 1% KVR condition than in the bottom 1% KVR. The failure to select eDNA shed from a fish school in the bottom 1% KVR is likely what caused the materialization to fail. Unlike Case2-1, the top 1% KVR in Case3-1 could not materialize a fish school (Figure 4). Even under the top 5% KVR, the fish schools materialized in Case2-1, Case2-2, Case2-3, and Case2-5, but not in Case3 (data not shown). It depends on whether the fish school is located in the surface or bottom layer of the bay. The tidal residual current in the bottom layer of Jinhae Bay is slower than that at the surface (Park et al., 2021). This indicates that the transport of eDNA copies shed from the fish school in Case3 was slower than that in Case2, and there were more eDNA copies in Case3. The fish densities around the fish school in Case3 were overestimated and were treated as outliers. Case3 was, therefore, not capable of materializing a fish school. We further checked that the scatter points of latent fish densities of 10-15 ind. m^{-3} (i.e., fish school) in the top and bottom 1% KVR of Case3-1 were eliminated as outliers (Figure 5). The R values increased with increasing KVR, and were 0.85, 0.84, and 0.82 in the top 90% KVR of Case1-1, Case2-1, and Case3-1, respectively; it was 0.87 in the bottom 90% KVR for all three cases. The improvement in the estimation of fish distribution according to the increase in KVR is clearly shown in Figure 3 and Figure 4. The fish school materialized at and above 70% KVR condition in all cases, and the clarity gradually increased.

The comparison results of the estimated fish density histogram and scatter plot with the those of the latent condition are shown in Figure 5. The histograms have been plotted as a bar graph with an interval of 0.5 ind. m^{-3}. The histogram count for 0-0.5 ind. m^{-3} in the top 1% KVR for Case2-1 was 3,320 higher than 435 observed for the latent. This was because the fish densities were underestimated below 0 ind. m^{-3} and were set to 0 ind. m^{-3}. The count between 2 and 5 ind. m^{-3} in the top 1% KVR for Case2-1 was 5,170, and that for the latent was 9,213. The count estimated over 15 ind. m^{-3}, which did not exist in the latent, was 1,004. As a result, this approach may underestimate and/or overestimate fish density under low KVR conditions. These issues were resolved as the KVR

219 increased. In the top 90% KVR of Case2-1, the count for 0-0.5 ind. m^{-3} and that over 15 ind. m^{-3} decreased to
220 1,082 and 49, respectively, and the count between 2 and 5 ind. m^{-3} increased to 8,318. We also confirmed the
221 improvement in agreement between estimated and latent fish densities according to the increase in KVR using the
222 scatter plots. These above-mentioned results were confirmed for other cases as well.
223

224 ## 4. Discussion

225 In recent years, eDNA approaches have been applied to studies on aquatic ecology and have shown their
226 potential to quantify aquatic organisms. To the best of our knowledge, the eDNA approach proposed by Fukaya et
227 al. (2021) is the most advanced method to estimate fish abundance. However, the eDNA approach has several
228 limitations (e.g., stationarity of fish population and homogeneity of eDNA shedding rate), and it may make bias in
229 the estimation of fish distribution. In their study, the fish distribution was not estimated reasonably, and they
230 discussed that one of the reasons was that the number of eDNA samples was relatively smaller than the number of
231 model grid cells. That means the number of eDNA samples is one of major factor to determine the reasonability
232 of estimating fish distribution. A quantitative evaluation of relationship between the number of eDNA samples and
233 reasonability of the eDNA approach is needed, but the eDNA research is in its early stages and research materials
234 are insufficient. Therefore, we conducted a numerical study to evaluate the eDNA approach according to the
235 number of eDNA samples. Specifically following steps, 1) randomize latent fish density and calculate eDNA
236 concentration from the tracer model and latent fish density; these values represent in situ value, 2) select cells and
237 assumed that we know eDNA concentration in only the selected cells; this process represents an eDNA sampling,
238 3) estimate the fish abundance and spatial distribution following the eDNA approach, 4) finally, evaluate the eDNA
239 approach by comparing between latent fish density and estimated one.

240 From the numerical evaluation, we revealed that the eDNA approach can reasonably estimate the fish abundance
241 regardless of the number of eDNA samples, if outliers of the fish density estimates are eliminated. The
242 reproducibilities of all cases before outlier elimination were overestimated to 1.194-1.869 under 50% KVR
243 condition, which were close to 1 after outlier elimination, with values being 0.913-0.960. This overestimation of
244 fish abundance was in line with the study by Fukaya et al. (2021). The reproducibility of fish abundance estimated
245 by Fukaya et al. (2021) was improved from 2.108 to 1.420 after omitting the cells near fish market in which the
246 fish density extremely high; we expect that the reproducibility could be further improved by eliminating outliers.
247 These results imply the outlier elimination is one of key factor to improve the reproducibility of abundance

estimation, and studies to quantitatively evaluate the relationship between outlier elimination method and reproducibility would be needed.

We figured out that reasonable estimation of fish distribution requires identification of 70% or more eDNA concentrations relative to the study area, but this is practically impossible. Extensive eDNA sampling requires more costs than the conventional methods, and it is worthless in an engineering. Estimation of fish distribution is hard pressed because it should be clearly accounted for physicochemical processes of eDNA (e.g., shedding, degradation, advection, diffusion, settling, and resuspension) in the whole study area (Andruszkiewicz et al., 2019; Fukaya et al., 2021). Those processes have been studied experimentally (Jane et al., 2015; Nukazawa et al., 2018; Sansom & Sassoubre, 2017; Sassoubre et al., 2016; Shogren et al., 2017; Wood et al., 2021), however, to expand few eDNA samples to the whole area is fully in a different category. Thus, in application of the eDNA approach to estimate a fish distribution, additional models, which could expand few eDNA samples to inference over the whole study area, may be required. Meanwhile, Shelton et al. (2022) demonstrated to expand few eDNA samples to the whole study area using a Bayesian state-space model for modelling eDNA concentration in the coastal ocean; in the study, the eDNA concentration was defined as a function of spatial coordinates and sample depth. Combination of the Bayesian state-space model with the eDNA approach proposed by Fukaya et al. (2021) may improve the reasonability of fish distribution estimation.

5. Conclusion

We evaluated the eDNA approach proposed by Fukaya et al. (2021) for estimating the abundance and spatial distribution of fish, based on a numerical study considering the number of eDNA samples relative to the study area as a simulation condition. The estimated abundances showed high reproducibility between 0.818 and 1.047 (if perfectly matched, reproducibility is 1), regardless of the number of eDNA samples. The approach reliably estimated the abundance, even with a small number of eDNA samples, if outliers of the fish density estimates are eliminated; however, this was not the case for the estimation of fish distribution. If the number of eDNA samples relative to the study area was lower than 10%, the correlations between estimated and latent fish densities were in a range of 0.01-0.20, and accordingly, were not able to materialize a fish school. The fish school was materialized only for Case2-1 even under the top 1% KVR condition; however, this is a particular case for pelagic fish and biased sampling. To obtain a correlation of over 0.6 and to materialize the fish schools regardless of fish distribution and eDNA sampling bias, it is necessary to know 70% or more eDNA concentrations relative to the

study area. Therefore, it is necessary to explore other methodologies for broadly estimating eDNA concentrations with fewer samples or for estimating fish distributions in a point-to-point manner (i.e., estimating fish density at eDNA sample points). Nevertheless, this eDNA approach is useful for enhancing our ability to estimate fish abundance in semi-enclosed bays. We expect our results to provide researchers with insights into the estimation of the abundance and spatial distribution of fish using eDNA.

AUTHORS CONTRIBUTIONS

designed research – S.P and S.Y.

performed research – S.P. and K.K.

contributed new reagents or analytical tools – S.Y. and K.K.

analyzed data – S.P. and S.Y.

wrote the paper – S.P. and K.K.

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Data Accessibility Statement

Data produced for this study are archived in Dryad (https://doi.org/10.5061/dryad.v9s4mw710) and will be available after manuscript acceptance.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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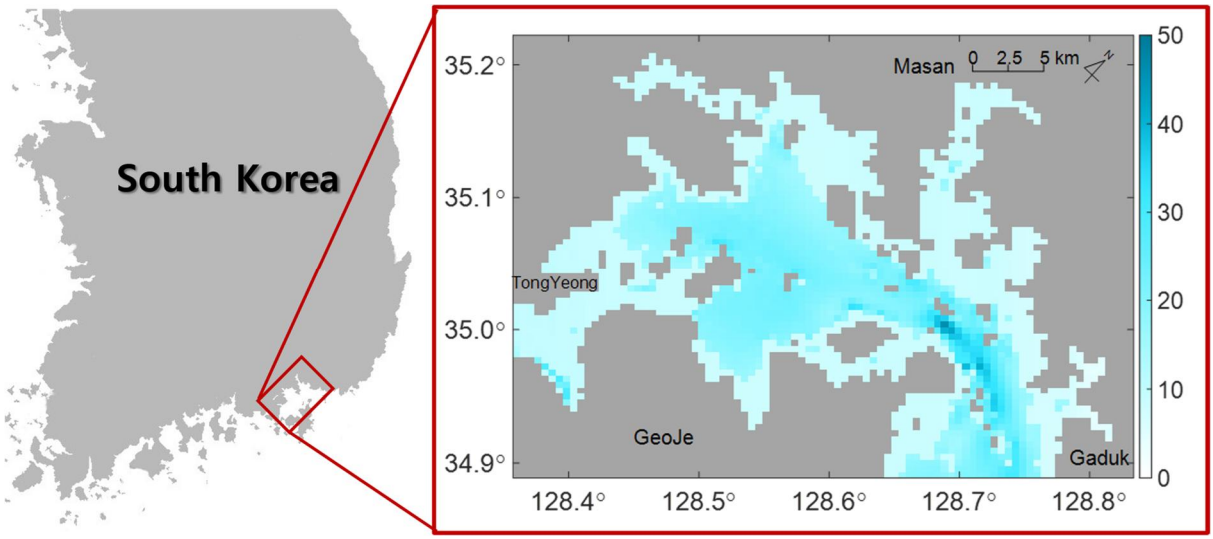
Table 1. Evaluation of estimated fish abundance (reproducibility) and distribution (R) in top and bottom 1%, 50%, and 90% KVR conditions for all cases.

	Reproducibility						**R between latent and estimated fish densities**					
	Top KVR			**Bottom KVR**			**Top KVR**			**Bottom KVR**		
	1%	**50%**	**90%**	**1%**	**50%**	**90%**	**1%**	**50%**	**90%**	**1%**	**50%**	**90%**
Case1-1	0.959	0.941	0.975	0.856	0.956	0.989	0.08	0.44	0.85	0.03	0.49	0.87
Case1-2	1.036	0.954	0.968	0.865	0.941	0.990	0.10	0.45	0.86	0.04	0.52	0.88
Case1-3	0.916	0.929	0.974	0.869	0.931	0.985	0.09	0.43	0.83	0.00	0.49	0.84
Case1-4	0.927	0.956	0.974	0.840	0.929	0.987	0.10	0.42	0.85	0.00	0.50	0.87
Case1-5	1.014	0.931	0.971	0.856	0.933	0.990	0.07	0.43	0.85	0.06	0.47	0.87
Case2-1	0.955	0.930	0.972	0.846	0.930	0.991	0.10	0.48	0.84	0.03	0.47	0.87
Case2-2	0.988	0.948	0.971	0.836	0.938	0.991	0.10	0.46	0.86	0.02	0.52	0.87
Case2-3	0.987	0.955	0.972	0.865	0.932	0.986	0.08	0.39	0.82	0.01	0.46	0.86
Case2-4	0.940	0.930	0.978	0.828	0.942	0.982	0.09	0.48	0.84	0.02	0.48	0.88
Case2-5	1.015	0.951	0.981	0.912	0.940	0.990	0.11	0.48	0.86	0.02	0.48	0.89
Case3-1	0.941	0.942	0.973	0.938	0.913	0.986	0.06	0.45	0.82	0.02	0.52	0.87
Case3-2	0.993	0.942	0.971	0.927	0.923	0.983	0.08	0.45	0.84	0.05	0.50	0.87
Case3-3	0.922	0.960	0.973	0.855	0.914	0.990	0.09	0.42	0.84	0.04	0.43	0.85
Case3-4	1.020	0.936	0.968	1.047	0.941	0.986	0.11	0.48	0.84	0.03	0.47	0.87
Case3-5	0.978	0.950	0.977	0.818	0.934	0.983	0.07	0.43	0.83	0.04	0.47	0.84
Mean	**0.973**	**0.944**	**0.973**	**0.877**	**0.933**	**0.987**	**0.09**	**0.45**	**0.84**	**0.03**	**0.48**	**0.87**
Std. Dev.	**0.039**	**0.011**	**0.004**	**0.059**	**0.011**	**0.003**	**0.02**	**0.03**	**0.01**	**0.02**	**0.03**	**0.01**

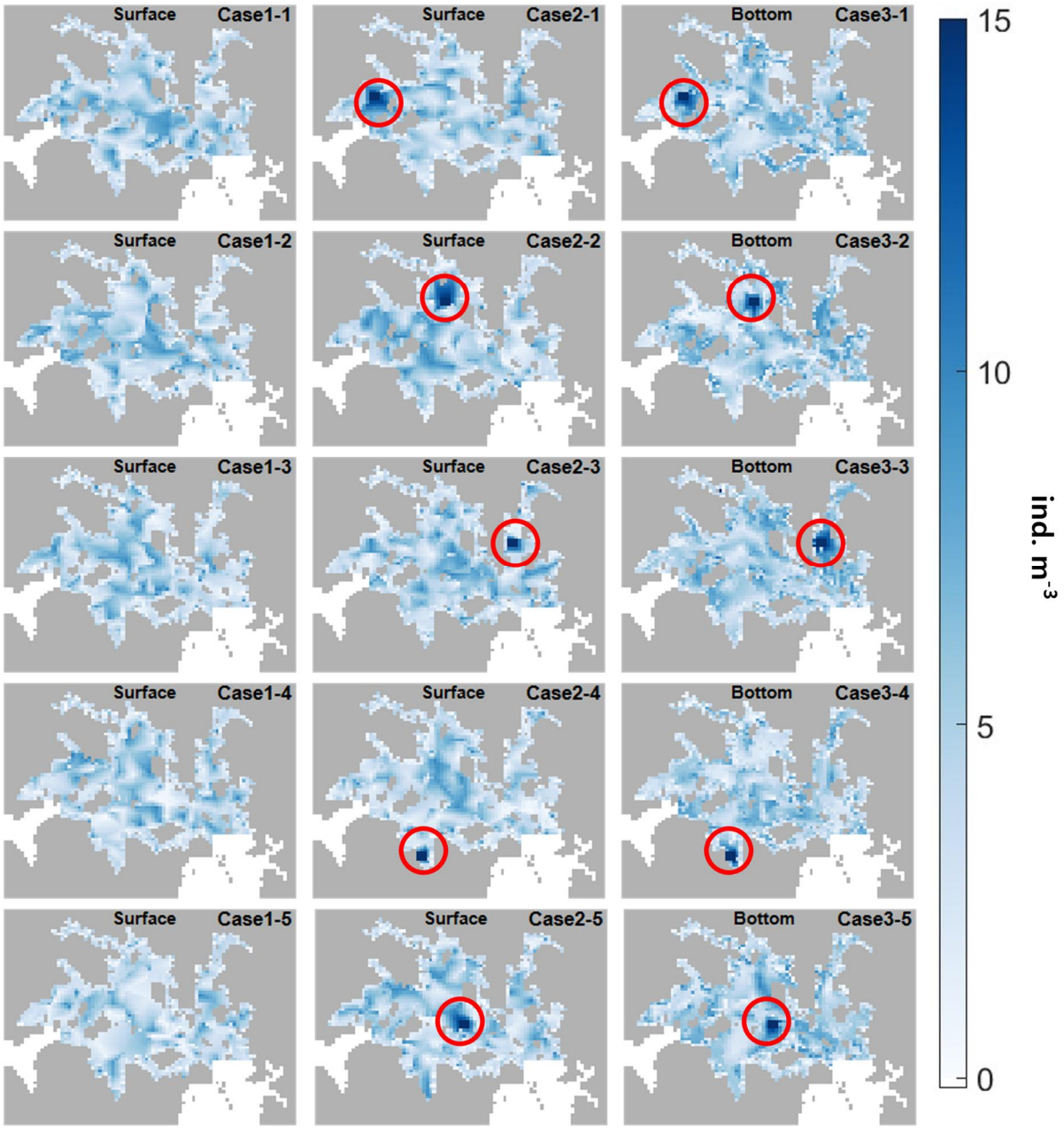
Table 2. Evaluation of fish abundance (reproducibility) and distribution (R) under all KVR conditions for Case1-1, Case2-1, and Case3-1.

		Case1-1		Case2-1		Case3-1	
		Abundance (Reproducibility)	R	Abundance (Reproducibility)	R	Abundance (Reproducibility)	R
Top KVR (%)	1	2.83e+10 (0.959)	0.08	2.89e+10 (0.955)	0.10	2.81e+10 (0.941)	0.06
	3	2.92e+10 (0.989)	0.10	2.95e+10 (0.974)	0.13	2.85e+10 (0.953)	0.10
	5	2.82e+10 (0.955)	0.13	2.91e+10 (0.961)	0.14	2.72e+10 (0.910)	0.14
	7	2.82e+10 (0.955)	0.14	2.83e+10 (0.935)	0.16	2.70e+10 (0.903)	0.16
	10	2.86e+10 (0.970)	0.16	2.85e+10 (0.944)	0.20	2.73e+10 (0.914)	0.19
	20	2.83e+10 (0.960)	0.23	2.74e+10 (0.905)	0.28	2.75e+10 (0.921)	0.24
	30	2.76e+10 (0.937)	0.32	2.75e+10 (0.910)	0.34	2.79e+10 (0.934)	0.30
	40	2.78e+10 (0.943)	0.38	2.80e+10 (0.925)	0.40	2.81e+10 (0.939)	0.37
	50	2.78e+10 (0.941)	0.44	2.81e+10 (0.930)	0.48	2.82e+10 (0.942)	0.45
	60	2.80e+10 (0.948)	0.54	2.78e+10 (0.920)	0.58	2.84e+10 (0.952)	0.52
	70	2.86e+10 (0.969)	0.64	2.87e+10 (0.949)	0.66	2.86e+10 (0.958)	0.62
	80	2.80e+10 (0.948)	0.72	2.87e+10 (0.949)	0.73	2.84e+10 (0.949)	0.70
	90	2.88e+10 (0.975)	0.85	2.94e+10 (0.972)	0.84	2.91e+10 (0.973)	0.82
Bottom KVR (%)	1	2.52e+10 (0.856)	0.03	2.56e+10 (0.846)	0.03	2.80e+10 (0.938)	0.02
	3	2.79e+10 (0.947)	0.01	2.47e+10 (0.818)	0.05	2.70e+10 (0.905)	0.05
	5	2.71e+10 (0.918)	0.07	2.64e+10 (0.874)	0.08	2.78e+10 (0.930)	0.05
	7	2.68e+10 (0.907)	0.10	2.71e+10 (0.895)	0.05	2.74e+10 (0.918)	0.09
	10	2.74e+10 (0.928)	0.12	2.80e+10 (0.926)	0.07	2.68e+10 (0.898)	0.11
	20	2.68e+10 (0.908)	0.21	2.72e+10 (0.898)	0.17	2.72e+10 (0.910)	0.23
	30	2.76e+10 (0.934)	0.30	2.73e+10 (0.902)	0.27	2.65e+10 (0.888)	0.33
	40	2.76e+10 (0.936)	0.41	2.74e+10 (0.904)	0.37	2.70e+10 (0.904)	0.43
	50	2.82e+10 (0.956)	0.49	2.81e+10 (0.930)	0.47	2.73e+10 (0.913)	0.52
	60	2.83e+10 (0.959)	0.59	2.87e+10 (0.948)	0.60	2.78e+10 (0.931)	0.61
	70	2.87e+10 (0.972)	0.68	2.91e+10 (0.962)	0.71	2.84e+10 (0.952)	0.70
	80	2.87e+10 (0.974)	0.79	2.96e+10 (0.979)	0.79	2.89e+10 (0.967)	0.79
	90	2.92e+10 (0.989)	0.87	3.00e+10 (0.991)	0.87	2.95e+10 (0.986)	0.87

453 

454 **Figure 1.** Isobathymetric map of Jinhae bay in South Korea.

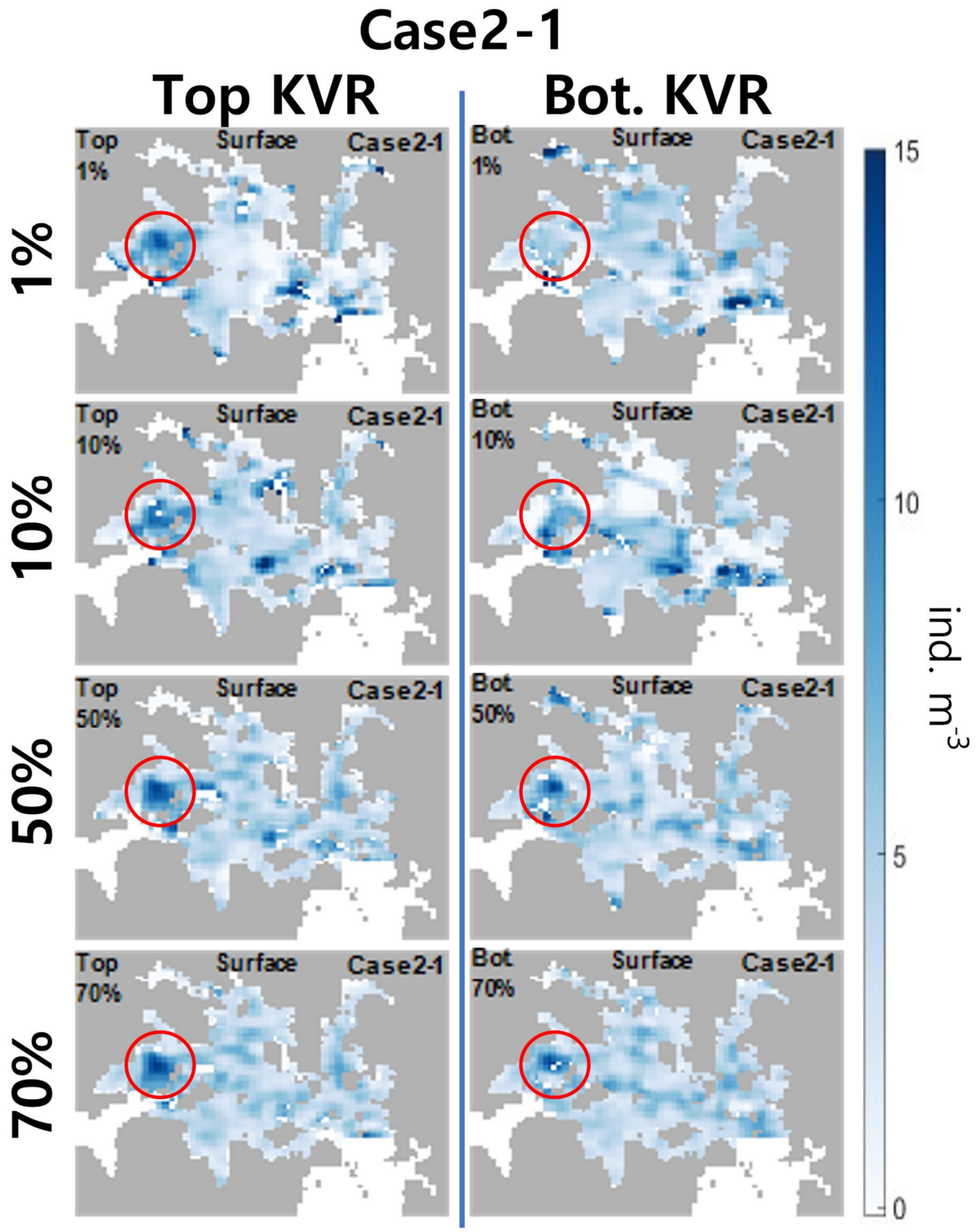
455



456

457 **Figure 2.** Latent fish distributions in surface layer of Case1 and Case2 and bottom layer of Case3. The red circle
458 represents a fish school with a relatively high fish density.

459



460

461 **Figure 3.** Estimated fish distributions of Case2-1 with KVR values of 1%, 10%, 50%, and 70% (red circle: fish
462 school).

463

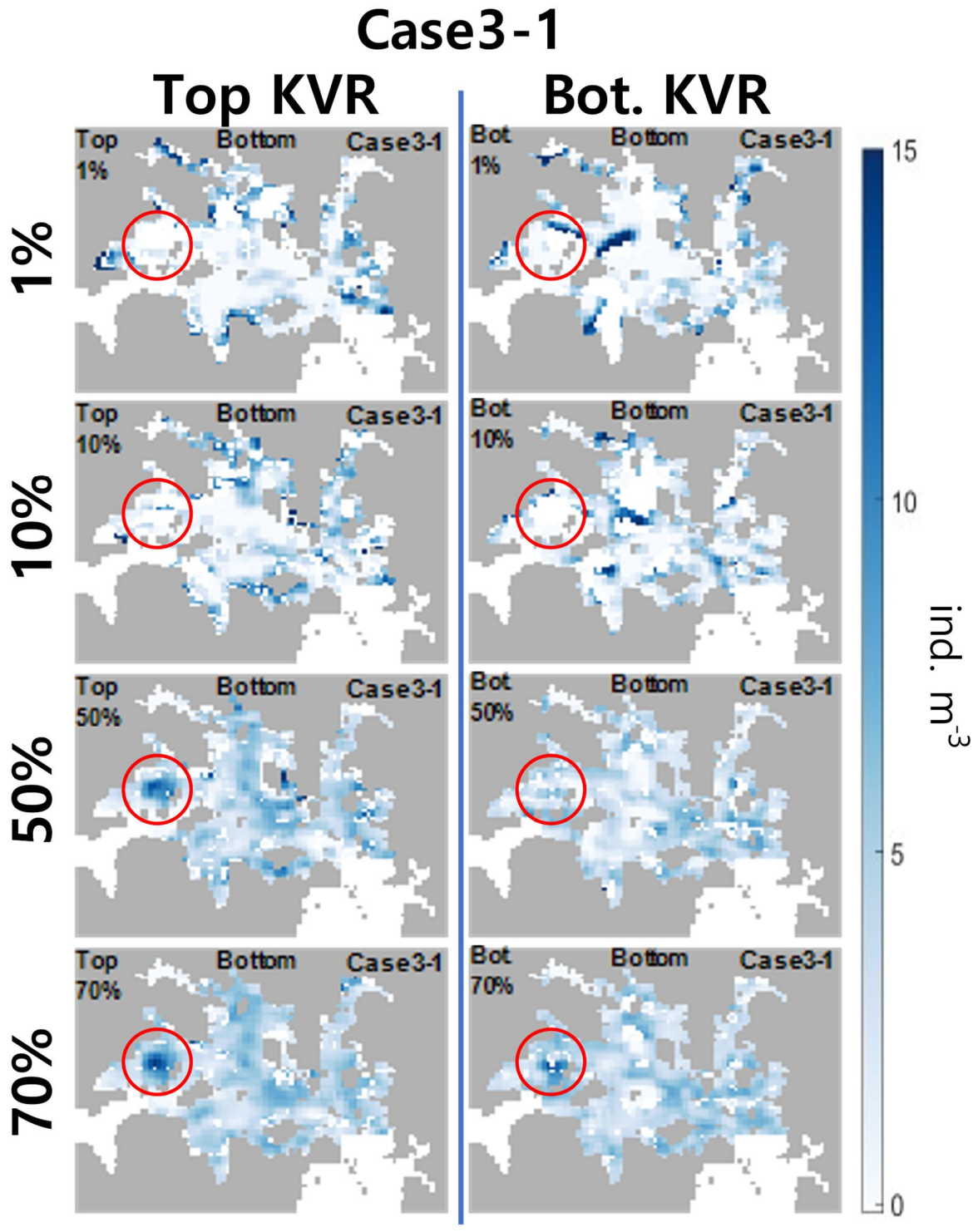


Figure 4. Estimated fish distributions of Case3-1 with KVR values of 1%, 10%, 50%, and 70% (red circle: fish school).

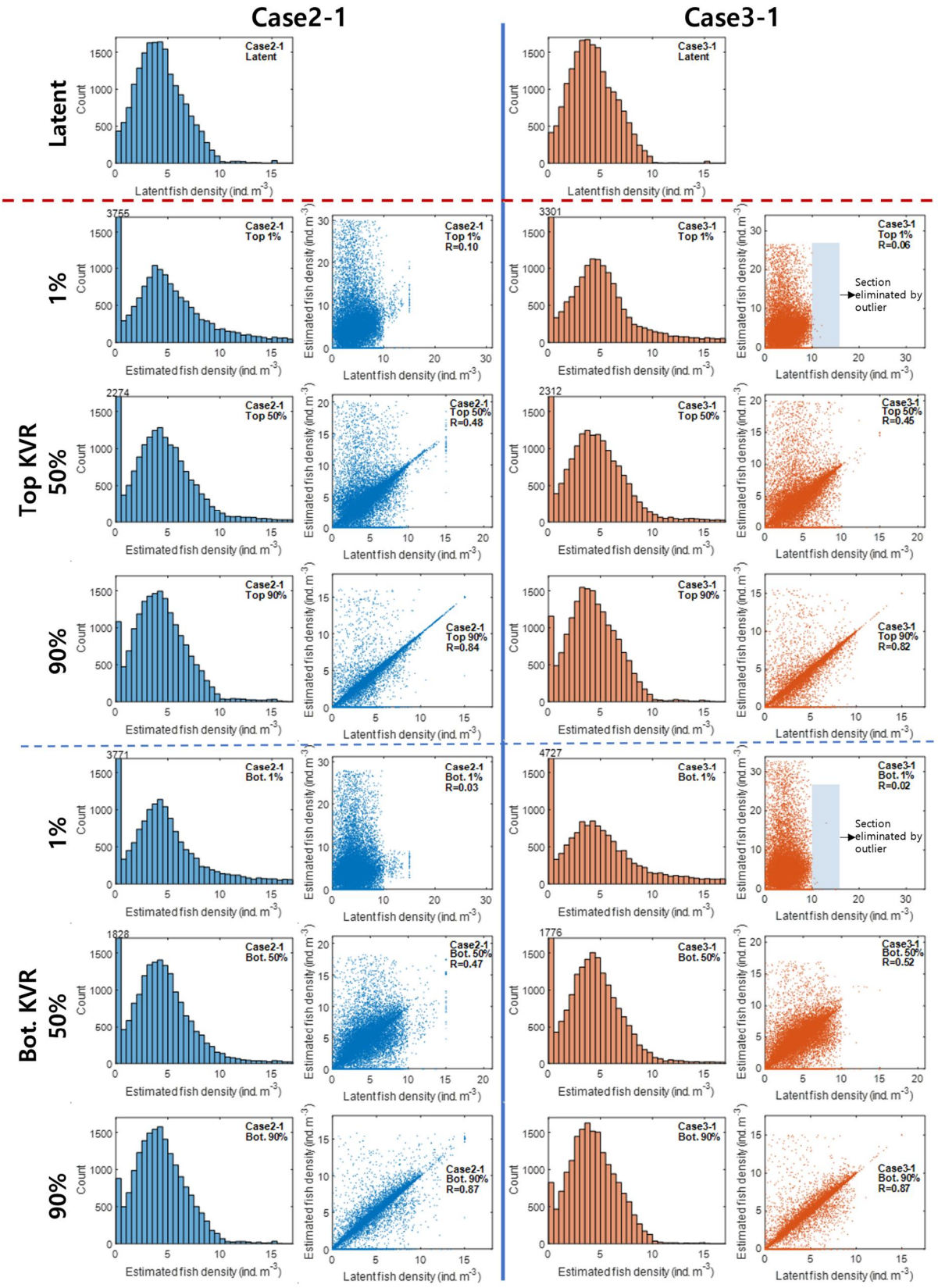


Figure 5. Histograms and scatter plots of fish densities of the Case2-1 and Case3-1 with KVR values of 1%, 50%, and 90%.