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A case study of time-series regression modeling: risk factors for pond-level mortality of farmed grass carp (*Ctenopharyngodon idella*) on a southern Chinese farm

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Abstract

Limited research has been done using multivariable statistical methods to assess factors associated with fish mortality in warm-water finfish aquaculture in China. We carried out a case study to test the hypothesized association between pond-level daily mortality of farmed grass carp and predisposing environmental and husbandry factors. Based on logbook data from a single farm in Guangdong province (China) in 2013, two-stage time-series regression (TSR) analyses were conducted to estimate the lagged effect of these predisposing factors on grass carp mortality. Factors assessed included temperature fluctuations, movement of fish into and out of ponds, and 3 types of treatments (antibiotics-antiparasitics, traditional Chinese medicine-probiotics, and chemicals to improve water quality). First, coefficients were estimated using a generalized linear negative-binomial model for each pond, and these coefficient estimates were combined using meta-analytic techniques. Sensitivity analyses were done to compare effects of changes in the 3 modeling components: distributional forms, number of spline knots, and types of autocorrelation terms. Model results in the case study indicated 2 risk factors might be associated with increased mortality of grass carp: (1) movements-in of new fish during the previous 14 days; and (2) increasing water temperature during the previous 7 days. Sensitivity analyses indicated good consistency of the estimates with different modeling components. Our findings highlight the utility of assessing daily farm records using TSR to develop hypotheses about potential risk factors for grass carp mortality in China.

Key words: Time-series regression; grass carp; mortality; risk factors; daily records.

1. Introduction

Grass carp (*Ctenopharyngodon idella*) is one of the most frequently farmed warm-water species in China due to its ease of domestication and acceptance in the marketplace (Cao et al., 2007; FAO, 2016). Despite the vast size of the industry, there are few field studies dedicated to systematic analysis of routinely-collected farm data from grass carp aquaculture (Yang et al., 2013). This is likely due to the lack of farm recording practices in this industry (Li et al., 2016; Jia et al., 2016).

Acute or chronic mortality events in pond aquaculture systems are not always fully investigated, making it difficult for producers to target specific control or prevention strategies that address fish losses. Analysis of mortality patterns can be a useful tool to understand potential causes of losses (Soares et al., 2011; Alba et al., 2015). For example, analyses can identify seasonal trends in mortality or patterns that coincide with particular management strategies.

In Asian aquaculture settings, where there is limited access to and use of disease diagnostic services, mortality could signal fish health problems caused by multiple factors, and analysis of mortality patterns and whether they correlate with specific events on farms can help inform potential control strategies (Tan et al., 2006; Bondad-Reantaso and Subasinghe, 2008; Serfling, 2015). For example, many agricultural production systems use all-in-all-out management to reduce the risk of introducing pathogens and/or naïve animals into existing animal populations. This is not well accepted in pond aquaculture for a number of reasons, most of which are logistical (Lin and Peter, 1991); however, the risk of mortality associated with not implementing this practice is not known, and could be determined if producers maintained information on fish movements and mortality (Boerlage et al., 2017).

Data extracted from daily records are well suited for the analysis of temporal associations by time-series regression (TSR) methods, which combine the concepts of ordinary regression and time series analysis to allow exploration of associations of outcomes with time-varying factors, such as management interventions or changes in temperature (Bhaskaran et al., 2013, Bernal et al., 2017). Although widely described, investigated analytically and applied in environmental epidemiology and public health intervention studies (Bell et al., 2004; Zeger et al., 2006; Imai et. al., 2015; Bernal et al., 2017), TSR has had limited use in animal health studies (Lloyd et al., 2000; Levine and More, 2009; Dórea et al., 2012; Lee et al., 2013). There are two recent publications involving TSR analyses in farmed aquatic animals (Gustafson et al., 2016; Piamsomboon et al., 2016), but no previous studies on warm-water finfish.

In this study, we examined the feasibility of using TSR methods to assess the association between time-varying risk factors and daily mortality counts of grass carp in multiple ponds from a farm in Guangdong province, China. We specifically targeted factors that may be associated with grass carp mortality: (1) ambient temperature; (2) handling (movement-in and movement-out); and (3) treatments.

2. Materials and methods

2.1. Data source and data entry

Data used in the study were daily pond-level records from 14 grass carp ponds located on the same farm, during a production cycle of grass carp in 2013. The farm was managed by a domestic aquatic feed company and used as a demonstration site for clients to learn about management practices in fish farming. All 14 ponds included in the study were in the first year of production. In addition to grass carp, these ponds held crucian carp *(Carassius*

carassius), silver carp (*Hypophthalmichthys molitrix*), and spotted silver carp (*Aristichthys nobilis*), but we did not include mortality data from these species.

The original logbook data for each pond were recorded on paper by staff working for an aquatic feed company. The following data were entered into Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) from the daily records (logbooks): (1) mortality counts (observed number of dead grass carp, but with no diagnosis or ascribed information on the cause of mortality); (2) movement-in and -out of fish (weight and size of fingerlings or new adult fish of multiple species); (3) treatment (chemical name and dose); and (4) water quality measurements (temperature, pH, and ammonia, etc.). Quality control of data entry was supervised by feed company personnel.

2.2. Description of variables

The outcome variable in this study was the daily mortality count of grass carp on each day for each pond. The number of grass carp on day 1, when movement-in was calculated, was based on fingerling size and total weight. After day 1, the grass carp number on any given day was obtained by subtracting the daily mortality from the total number of grass carp on the previous day.

Seven predictor variables were assessed for their acute or delayed associations with fish mortality. Except for temperature, all movements and treatments of fish were coded as binary (dichotomous) variables. The 3 variables related to movement-in of new fish and movement-out of fish were defined as follows. (1) *mi3d*: whether there was movement of fish into the pond during the previous 3 days. We expected to find an increase in mortality soon after the movement-in of fish if mortality was associated with poor environmental conditions, due to increased biomass or from a peracute infectious disease. (2) *mi2w*: whether there was movement-in of fish during the previous 14 days. This is the time frame we anticipated would be required for pathogen introduction associated with a transfer of fish to influence mortality counts. (3) *mo3dm*: whether there was movement-out of fish during the previous 3 days, except when the pond was within 10 days of final harvest. Movement-out of fish from grow-out ponds of grass carp was hypothesized to cause acute mortalities due to over-crowding and stress during the harvest procedures.

Three variables related to treatments were used to estimate the change in fish mortality after treatments: (1) *atbp7d*, whether antibiotics or antiparasitics were used during the previous 7 days; (2) *ctpr7d*, whether Chinese traditional medicine or probiotics were used during the previous 7 days; and (3) *wimp3d*, whether chemicals to improve water quality were used during the previous 3 days. The chemicals most frequently used for water quality treatment were povidone-iodine, calcium hypochlorite, copper sulfate, and chlorine dioxide.

Temperature was measured by *tmax06***,** a continuous variable, indicating the 7-day average maximum daily atmospheric temperature. All historical records of atmospheric temperature for the study area were retrieved from online open-source meteorological data available on the official website of Guangzhou City Meteorological Information Centre (http://www.gz121.gov.cn/gywm/sjkf/).

2.3. Exploratory descriptive analysis

We summarized production information for each pond, including movement-in and movement-out dates, grass carp mortality, frequencies of movements and treatments, and ambient temperature fluctuations. Frequency distributions were used to explore the association between binary predictors and to facilitate the understanding of how treatment practices were related, i.e. single methods, or combinations of 2 or 3 treatments. Group means of atmospheric temperature (*tmax06*) were also compared for days when the value for each binary variable was equal to 1 (*atbp7d, ctpr7d* and *wimp3d*) and days when it was equal to 0. Sign tests and generalized estimating equations were also carried out, as detailed in supplementary materials 1 (S1).

2.4. Two-stage time-series regression (TSR)

We used the two-stage TSR analysis (Dominici et al., 2000) to assess risk factors of grass carp mortalities. All modeling steps were implemented in Stata 13 (Stata Corp., College Station, TX, USA). In the first stage, the series of daily grass carp mortality counts for each pond were analyzed separately by generalized linear models. For these models, the distributional form, the modeling of temporal effects, and incorporation of autocorrelation were first investigated in exploratory analyses. In order to obtain the most meaningful comparison across ponds (i.e., in the second stage of the modeling) it was preferable to use the same models for all ponds. On the other hand, computationally complex models may not be equally suited for all ponds and, in extreme cases, models that are too complex may fail to produce meaningful estimates within ponds. Excluding certain ponds from analysis due to computational problems would likely lead to selection biases, so our guiding principle for selecting appropriate within-pond models was to enable sufficient flexibility to capture the most important features of the data while allowing for estimation in all ponds. The robustness and impact of different choices for among-pond modeling was explored by a sensitivity analysis.

The wide variability of within-pond counts led us to consider negative binomial instead of Poisson models. We adjusted for the population-at-risk by including a logarithmic transformation of total number of fish as an offset (as implemented in the *glm* command in Stata).

The possible fluctuations of outcome counts over time due to unmeasured factors were explored using a smooth cubic spline function with varying numbers of knots (Bhaskaran et al., 2013). We initially evaluated between 2 and 9 knots, but due to convergence problems at the pond level when many knots were included, we restricted our models to splines with 5 and 6 knots. Adjustment for autocorrelation was done by including both 1-week and 2-week lagged deviance residual terms, as described above, in the predictive part of the model (Brumback et al., 2000).

In the second stage of each TSR model, the estimated coefficients and standard errors were the results of the first-stage analysis (for each predictor obtained from the analysis of each individual pond) and were combined using a random-effects meta-analysis (Borenstein et al., 2009). Forest plots were used to depict the variability in predictor estimates across ponds, and their consistency was reflected in the 95% confidence intervals.

We compared the results of the two-stage TSR analysis to those based on different within-pond models. In addition, we also compared results obtained for a multivariable analysis, including all 7 predictors simultaneously, and separate analyses including a single predictor at a time (together with other model terms). Based on descriptive and final model results, we investigated the potential for confounding by some of the predictors by comparing the results of the selected model to those without chosen combinations of the predictors involved. Details on main model selection and sensitivity analysis can be found in supplementary materials 2 and 3 (S2 & S3).

3. Results

3.1. Exploratory descriptive analysis

3.1.1. Production information

Start and finish dates for the production cycle in the 14 ponds varied, with the earliest movement-in date in January 2013 (ponds 9 and 10), and latest movement-in date in April 2013 (pond 33) (Table 1). The mortality count pattern and the frequency of non-zero mortality days differed across ponds (Table 1). The 5 highest mortality counts were reported from ponds 10, 11, 12, 19, and 33. Between 32% and 80% of observations had zero mortality in each pond (Table 1), suggesting that at least some of the ponds had excessive zero mortality counts.

3.1.2. Descriptive analysis of predictor variables.

Ambient temperature was considered a proxy for water temperature because the latter data were incomplete. Based on fluctuation patterns of daily water and atmospheric temperature, we found that daily water temperatures were similar overall to atmospheric temperatures (Fig. 1).

Frequencies of management practices for each pond are summarized in Table 2. For movements of fish, all 14 ponds experienced multiple movements-in, but not all ponds were harvested multiple times. No movements-out of fish occurred in 3 ponds during the study period (ponds 21, 23, and 24), and movements-out of fish were recorded only once for 3 other ponds (ponds 11, 22, and 33) (Table 2). For antibiotic and/or antiparasitic treatments of fish, most ponds had at least one of each of these treatments applied during the study period, with the exception of no antiparasitic treatments in ponds 13, 15, and 33. Applications of Chinese medicine, probiotics, and chemicals to improve water quality were more frequent than antibiotic and/or antiparasitic treatments across all ponds (Table 2).

The simultaneous use of 2 treatment groups, traditional Chinese medicine-probiotics (*ctpr7d*) and water quality treatments (*wimp3d*), was common in all ponds. Antibiotics and antiparasitic treatments were rarely combined with traditional Chinese medicine-probiotics, except in pond 11. Both traditional Chinese medicine-probiotics and water quality treatments were likely to occur during days with higher atmospheric temperatures. We have illustrated the above results with pond-11 data in Figure 2.

3.2. TSR modeling

For the first-stage analysis, we chose for the following components for the 7-predictor model: a negative binomial distribution (without zero-inflation), a 5-knot time spline, and two-lagged deviance residual terms. Pond 33 did not produce meaningful results for the first-stage TSR analysis. Exploration of the data suggested this was due to irregularly spaced missing data on fish mortality counts, so we excluded this pond from the TSR analysis. The estimates generated for each predictor, based on the chosen model, were applied to each of the 13 ponds (i.e., without pond 33). We summarized meta-analyses results for each predictor in the second stage in Table 3 and Figures 3 and 4.

Three predictors, movement into the pond within 3 days (*mi3d*), movement-out within 3 days (*mo3dm*), and the treatment with antibiotics-antiparasitics within 7 days (*atbp7d*), were not significantly associated with variations in mortality counts. Four predictors had significant or close to significant associations with the incidence of mortality across all ponds (Table 3), and the associations can be interpreted, in terms of incidence rate ration (IRRs), after adjustment for the time-varying predictors, as follows:

(1) Movement-in of fish (*mi2w*): the overall IRR of 2.01 (95% CI, 1.50 to 2.68) indicated that the incidence rate of pond-level mortality on days with movement-in of fish during the previous 14 days was estimated to be two-fold higher than on days without preceding movements. There was some between-pond heterogeneity in the fish movement effect (association with mortality count) ($p=0.035$, $I^2=45.9\%$), with one pond (20) showing an apparent beneficial effect, although with wide CI and outweighed by the adverse effects in all other ponds.

(2) Use of Chinese tradition medicine and probiotics (*ctpr7d*): the overall IRR of 0.69 (95% CI, 0.57 to 0.85) indicated that the incidence rate of pond-level mortality on days with a treatment with traditional Chinese medicine or probiotics during the previous 7 days was

about 1.45 (1/0.69) times lower than on days without such treatments during the previous 7 days.

(3) Use of chemicals to improve water quality (*wimp3d*): the IRR of 1.24 (95% CI, 1.03 to 1.48) indicated a slight increase in incidence on days with water quality treatments during the previous 3 days.

(4) Temperature (*tmax06*), the IRR of 1.17 (95% CI, 1.06 to 1.28) indicated a 1.2-fold increase in incidence for every 1^{0}C increase in temperature during the previous 7 days.

Detailed modeling options for the purpose of sensitivity analysis (Table S3) and their comparisons (Figs. S1-7) are in supplementary materials (S3). In the main model, high levels of heterogeneity across ponds, also referred to as inconsistency (Higgins et al., 2003), were found for *tmax06* (I^2 =78.7%). The estimates of the remaining 6 predictors had moderate heterogeneity, with I^2 ranging from 45.3% to 59.8%.

4. Discussion

To our knowledge, this is the first use of time-series regression analysis to investigate the association between common farm management strategies, such as movement of fish in and out of ponds, and mortalities of grass carp in China. Our study demonstrated the feasibility of TSR modeling of risk factors for fish mortality, which might be applicable in other warm-water aquaculture species. We also evaluated the usefulness of farm-records in grass carp aquaculture for identifying trends that may be associated with commonly-used management strategies, detailed below.

4.1. Movement-in of fish

Movement-in of fish within a 14-day period was significantly associated with increased mortality counts of grass carp on our study farm. In other words, a significant increase in mortality was found within 14 days of the introduction of fish, which suggested that, on average, movements had adverse impacts on fish, even though these changes might take up to two weeks to manifest. This result is what would be expected if the movement of fish into a pond introduced a pathogen and subsequent infection with an incubation period less than 2 weeks or if the new fish were exposed to a pre-existing pathogen in the pond (Barton, 2002). Unfortunately, we could not tell from the records whether the fish that died were new or resident fish; however, given the association found in this study, it may be worthwhile for future researchers to investigate whether the movement-in of fish is a potential pathway of infectious pathogens to fish already in the pond. The delayed mortality, post introduction of fish, could also suggest stress-related issues. More detailed investigation of the cause of mortality would help differentiate this from an infectious disease, which is important, as the control strategies for each would differ.

Mortality from sudden changes in water quality might occur acutely in pond aquaculture (Boyd and Tucker, 1998). The fact that we did not observe a change in mortality with movements of fish 3 days prior (*mi3d*) suggests, on average, ponds on this farm did not experience short-term water quality issues associated with fish movements.

Despite the issues that can arise from mixing fish populations, introductions of new fish into ponds and partial harvests of populations are common practices in carp aquaculture. All-in-all-out farming strategies have been shown to be effective in several food animal production systems in reducing the likelihood of disease outbreaks (Rimstad et al., 2006; Cox and Pavic, 2010), but these approaches might be difficult to apply in grass carp culture, given the industry's practice of multiple movements-in and multiple harvests, with the purpose of maximizing energy utilization in the pond ecosystem (Lin and Peter, 1991). Our study suggests producers may need to re-evaluate the practice of frequent movement-in of fish, as it was associated with increased mortality counts.

4.2. Treatment with traditional Chinese medicine or probiotics

In our study, this treatment was associated with reduced carp mortality and was significant in 4 of the ponds, as well as in our overall analysis. Traditional Chinese medicine treatments were usually administered together with probiotics and vitamin C in the feed, and were associated with a reduction in fish mortality. There have been studies to evaluate plant herbs as alternatives to antibiotics to treat fish disease (Pandey et al., 2012; Guo et al., 2014; Mo et al., 2016). Interestingly, the reason for the application of Chinese medicine in our study was not known, so we cannot say whether the fish had an infectious disease. However, it appeared that when these products were used on this particular site, fish mortality decreased.

Unlike the use of Chinese medicine, the use of antibiotics and/or antiparasitics 7 days prior was not associated with a decrease in mortality. It is possible that this group of pharmaceuticals were used prophylactically instead of as a therapy, in which case our results would suggest they were effective. However, given that the Chinese medicine was used therapeutically (i.e. we found a reduction in mortality with these products) it seems more likely that antibiotics were also used as a therapy.

According to the anecdotal note from the farm workers taking the records, antibiotics and/or antiparasitics were more likely to be used when mortalities were high. If these products were used as therapeutants, then our analysis suggests they were often ineffective at significantly reducing mortality. Antibiotics are only effective against bacterial pathogens, and not all products are broad spectrum, so if the farmer did not diagnose the specific cause of mortality prior to treatment it is possible the antibiotic was not an appropriate treatment. Given the mixed treatment results found in this analysis, farmers may benefit from investigating the specific causative pathogen responsible for mortality to identify appropriate treatment in the future.

4.3. Use of chemicals to improve water quality

In our study, this treatment was associated with increased rather than a reduction in fish mortality. According to anecdotal notes from fish farmers and fish veterinarians in China during our 2014 surveys (Jia et al., 2017), treatment of water with chemicals is more commonly applied to prevent the occurrence of disease or reduce mortality than other health management practices. However, due to the lack of diagnoses, farmers' decisions on water quality improvement relied on the guidance of fish health personnel, and treatments were usually done prophylactically, without determining whether poor water quality was an issue. Furthermore, water quality improvement may have adverse effects on pond biota (Pillay and Kutty, 2005), which might lead to degradation of the pond ecosystem and eventually result in adverse health events (Moll, 1986).

In general, chemotherapeutic treatments are applied to return mortality to normal baseline levels. However, treatments, in some cases, may not be effective because of misdiagnosis, resistance, improper dose usage, or other limiting factors. Endemic parasitic problems of finfish might compromise the integument of the fish and, hence, a chemical treatment of water might exacerbate mortality instead of reducing it, or may do nothing to interrupt the initial upward trend in mortality associated with the start of an infectious disease outbreak. The fact that we did not see a corresponding positive effect of water treatments suggests this producer should further investigate water quality parameters prior to applying the treatments.

4.4. Water temperature

The estimates of the association between water temperature and mortality in this study were relatively consistent regardless of the model components used, and were always statistically significant. The increasing trend in mortality associated with high daily water temperature suggests producers should further investigate management strategies that target this environmental factor.

Ambient water temperature and oxygen availability are the most influential environmental factors affecting aquatic organisms. We included temperature in our model to control for potential confounding effects on other risk factors, i.e. management practices. Absolute water temperature and changes in temperature are likely to have cumulative chronic effects on pond systems (Pickering, 1998). The upper lethal temperature range for grass carp is 33-41^{0}C, with a mean critical thermal maximum of 39.3^{0}C (Chilton and Muoneke, 1992). However, under intensive pond aquaculture, even within the normal range of water temperature for carp, survival rates of grass carp have been reported to be adversely associated with increased ambient temperatures (Song, 2012). Increases in water temperature may reduce the level of oxygen in the water and increase the demand for it, exacerbating the issue. High water temperatures might also alter ammonia concentrations and cause accumulations of this chemical and its metabolites in aquaculture systems (Alcaraz and Espina, 1995).

4.5. Time-series analyses

The use of multi-series multivariable TSR models allowed us to quantify the impact of multiple time-varying management factors, while controlling for extraneous slow changes in time and important specific time-varying confounders (e.g. temperature) and also accounting for heterogeneity between individual ponds in both outcomes and management variables. The TSR analysis demonstrated consistent associations, across ponds, of fish movements into ponds and of certain treatments, even though these associations were difficult to discern from simple descriptive statistics.

TSR methods could also apply to data from multiple farms, though the multi-level nature of the second-stage analysis (i.e. differences across farms) would need to be controlled. Several extensions of TSR beyond our application have been developed, and with large, informative datasets, in particular, it is possible to infer the lag structure of an association between a predictor and outcome directly from the data within the model (Schwartz, 2000), even if the association is non-linear (Gasparini et al., 2010; Gasparrini and Armstrong, 2013), rather than by construction of moving averages of exposure variables, as was done in this paper. Despite the utility of this type of analysis, especially for time-varying predictors such as treatments, few time-series studies have been used to assess aquatic animal health management strategies or risk factors (Chang et al., 2007; Lessard et al., 2007; Connors, 2011), perhaps due to the difficulties in accurately measuring fish mortality in the aquatic environment. Although it is difficult to accurately capture all mortality counts in earthen aquaculture ponds, the patterns observed in the subset can be useful for informing producers of potential impacts of management over time.

4.6. Study scope and limitations

First, the major limitation in this study was the quantity of data available to us. Out of more than 100 grass carp farms that we visited in China between 2013 and 2014, we only identified one farm with sufficient recorded data to conduct this type of statistical analysis, which limited the external validity of our analysis. However, the study does highlight the potential benefits of record keeping on fish farms. To deal with the limited data we had to simplify some of our predictors. For example, we used binary predictors for management strategies, which resulted in a loss of information.

Second, the variable *tmax06,* denoting the average temperature of the previous week, was missing for the first 6 observations for each pond, so these observations were not included in the models. Since mortality immediately after the initial movement-into the ponds was not our main interest, we were not overly concerned that data for this period was missing.

Third, correlation between treatment predictors and *tmax06* was found to be high in most ponds. Traditional Chinese medicine and probiotics were often found to be used simultaneously with other treatments, and these correlations made it difficult to discern the associations of individual predictors. The simplification of our predictors and the confounding of some management practices may have affected the model estimates, so we were conservative in our inferences. However, we believe that TSR modeling will be useful for future risk factor studies in grass carp aquaculture.

5. Conclusions

To our knowledge, this is the first application of TSR to a risk factor study of daily mortalities of warm-water finfish. Our results indicate that movement of fish into ponds, use of chemicals to improve water quality, and high daily temperatures were associated with increased mortality of grass carp, while treatments using traditional Chinese medicine and probiotics were associated with low mortality. Although generalization of these findings to other small-scale farm settings should be done with caution, the methods and modeling undertaken demonstrate the utility of daily record-keeping and analysis of those records. Our analyses also suggest that producers may benefit from investigating specific causes of mortality, as some of these events were associated with management strategies, which could be subsequently modified.

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Table 1 Stocking date, final date of production, and grass carp mortality counts summarized for each pond.

			All mortality counts					**Non-zero mortality counts**			
Pond	**Stocking date**	**Final record date**	Min	Max	Mean	Median	SD	Event frequency[a]	Mean	Median	SD
9	1/14/2013	6/23/2013	0	174	11.9	1	29	0.547	21.8	3	36.4
10	1/14/2013	9/24/2013	0	295	7.2	0	28.5	0.449	16.1	3	40.9
11	1/15/2013	9/24/2013	0	1620	84.7	11	177.4	0.569	148.8	73	214.1
12	1/16/2013	9/24/2013	0	300	9.2	0	28.7	0.44	20.9	6	40.5
13	1/17/2013	8/30/2013	0	73	4.4	2	8.3	0.681	6.4	3	9.4
14	1/19/2013	9/24/2013	0	63	9	1	13.3	0.522	17.3	15.5	13.9
15	1/17/2013	8/30/2013	0	76	4.7	2	9.7	0.633	7.5	4	11.4
19	3/14/2013	8/30/2013	0	411	11.8	0	50.5	0.465	25.4	3	71.9
20	3/15/2013	9/24/2013	0	81	2.6	0	8.8	0.345	7.6	3	13.7
21	3/26/2013	8/30/2013	0	95	10	1	20.8	0.551	18.1	5	25.3
22	3/25/2013	9/24/2013	0	68	3.7	0	8.9	0.495	7.5	4	11.5
23	3/26/2013	8/30/2013	0	212	11	0	35.2	0.43	25.6	8	50.3
24	3/25/2013	9/24/2013	0	41	5.4	1	7.9	0.522	10.4	9	8.3
33	4/29/2013	9/24/2013	0	870	38.9	0	111.9	0.201	193	144	182.1
Total			0	1620	16	0	66.9	0.498	32.2	6	92.1

Note: [a] Event denoted a day with mortality of grass carp more than zero. Denominator for the calculation is the number of days between stocking date and the final record date

Table 2 Frequencies of management variables: movements and treatments of fish and pond water.

Pond	Movement of fish		Treatment of fish or using of chemicals to improve pond water quality				
	Stocking	Harvest	Antibiotics	Antiparasitics	Traditional Chinese Medicine	Probiotics	Chemical for water quality improvement
9	9	5	15	2	26	14	35
10	7	3	7	3	30	24	41
11	3	1	37	2	58	15	37
12	7	9	3	3	25	15	36
13	5	3	5	0	37	17	41
14	6	5	1	6	31	19	40
15	5	4	2	0	28	17	39
19	6	6	5	3	15	18	21
20	6	3	3	2	14	19	24
21	4	0	0	2	20	17	31
22	4	1	2	2	27	21	36
23	4	0	11	3	17	14	34
24	4	0	2	2	35	26	40
33	8	1	13	0	30	14	40

Table 3 Estimated means and 95% confidence intervals (CI) of incidence rate ratios for seven predictors, combined by separate random-effects meta-analyses in the second-stage of a time-series regression analysis. The regression coefficients entered into the meta-analysis were extracted from individual analyses for each of 13 ponds by multivariable negative-binomial regression models that included 5-knot cubic spline functions of time and deviance residuals lagged one and two time steps as predictors.

Predictor variables and effects evaluated	**Incidence rate ratio**	**95% CI**	**P-value**
Movement of fish within previous 3 days (*mi3d*=1)	0.83	(0.57, 1.35)	0.46
Movement in of fish within previous 14 days (*mi2w*=1)	2.01	(1.50, 2.68)	<0.001
Movement out of fish within previous 3 days (*mo3d*=1)	1.37	(0.83, 2.26)	0.22
Treatment with antibiotics or antiparasitics within previous 7 days (*atbp7d*=1)	1.28	(0.97, 1.69)	0.08
Treatment with CTM or probiotics within previous 7 day (*ctpr7d*=1)	0.69	(0.57, 0.85)	<0.001
Water quality treatment within previous 3 day (*wimp3d*=1)	1.21	(0.99, 1.48)	0.06
Temperature of previous week increase by 1 ^{0}C (*tmax06)*	1.17	(1.06, 1.28)	<0.001

Figure legends (Figs. 1- 4)

Fig 1 Fluctuation of atmosphere temperature and recorded water temperature.

Note: 1. *temp* denoted water temperature measurement records in the data. Variation of water temperature among different ponds was assumed to be negligible. 2. *max_temp* denoted atmosphere temperature from online weather historical records for the study area 3. *Weather* denotes sunny with the value of 3, cloudy with the value of 2 and rain with the value of 1.

Fig 2 Occurrence of the daily observed mortality and the management practices recorded for that day in Pond 11.

Note: 1. Mort denotes the observed mortality of the corresponding day (shown as circles); 2. The codes for the 5 interventions are as follows: 1) move-in: movements-in of fish; 2) move-out: movements-out of fish; 3) atbp: treatment of antibiotics or antiparasitics; 4) ctpr: treatment of traditional Chinese medicine or probiotics; 5) wimp: using chemicals to improve water quality.

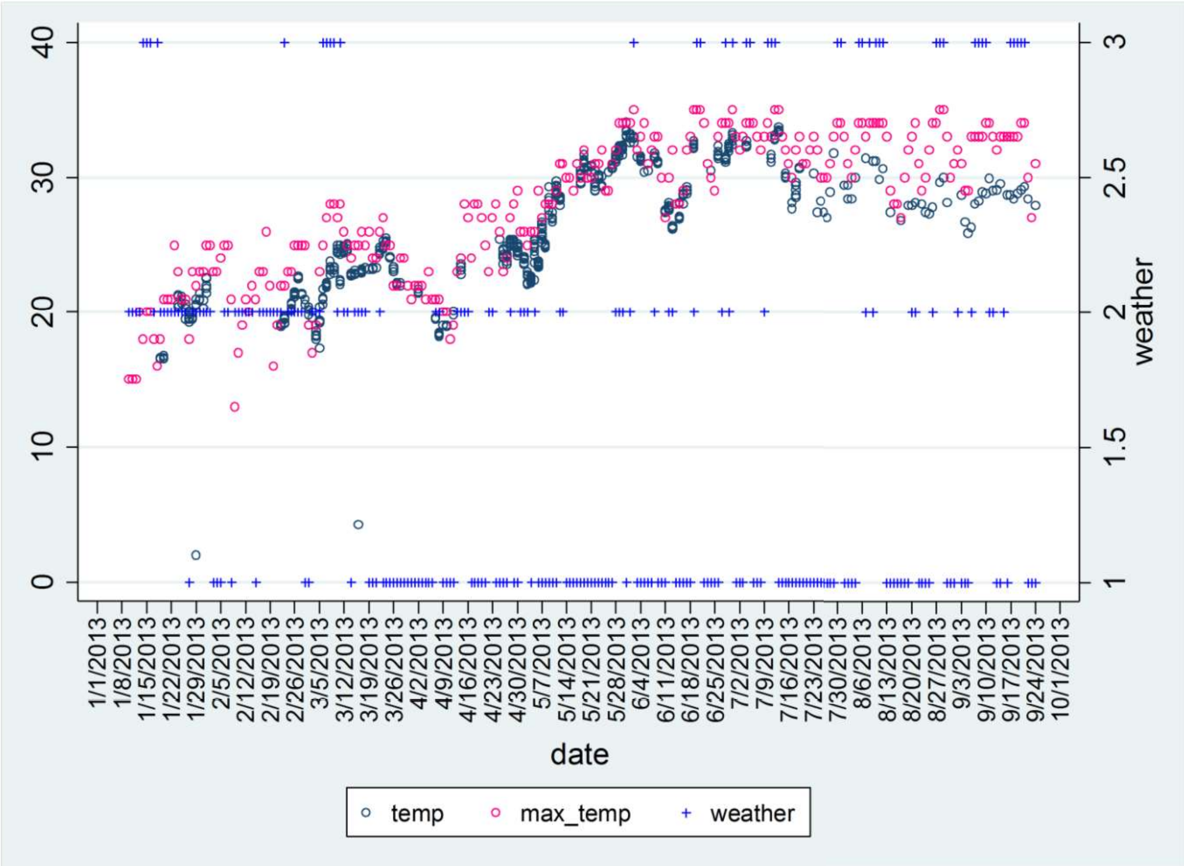
Fig 3 Forest plot for the random-effect estimates of movement-in of fish in the previous 2 weeks (*mi2w)* for the negative-binomial regression model across the 13 ponds [a].

Note: [a] The 13 ponds are ponds 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24 which are listed in ascending order. Pond 33 was omitted. [b] IRR = incidence rate ratio. [c] Overall I-square was reported as 45.9% with p-value of 0.035.

Fig 4 Forest plot for the random-effect estimates of treatment with CTM or antibiotics (*ctpr7d)* for the negative-binomial regression model across the 13 ponds[a].

Note: [a] The 13 ponds are ponds 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, and 24 which are listed in ascending order. Pond 33 was omitted. [b] IRR = incidence rate ratio. [c] Overall I-square was reported as 45.3% with p-value of 0.038.

Fig. 1.



0
10
20
30
40
1
1.5
2
2.5
3
weather
1/1/2013
1/8/2013
1/15/2013
1/22/2013
1/29/2013
2/5/2013
2/12/2013
2/19/2013
2/26/2013
3/5/2013
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3/19/2013
3/26/2013
4/2/2013
4/9/2013
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4/23/2013
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5/7/2013
5/14/2013
5/21/2013
5/28/2013
6/4/2013
6/11/2013
6/18/2013
6/25/2013
7/2/2013
7/9/2013
7/16/2013
7/23/2013
7/30/2013
8/6/2013
8/13/2013
8/20/2013
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temp
max_temp
weather

Fig. 2.

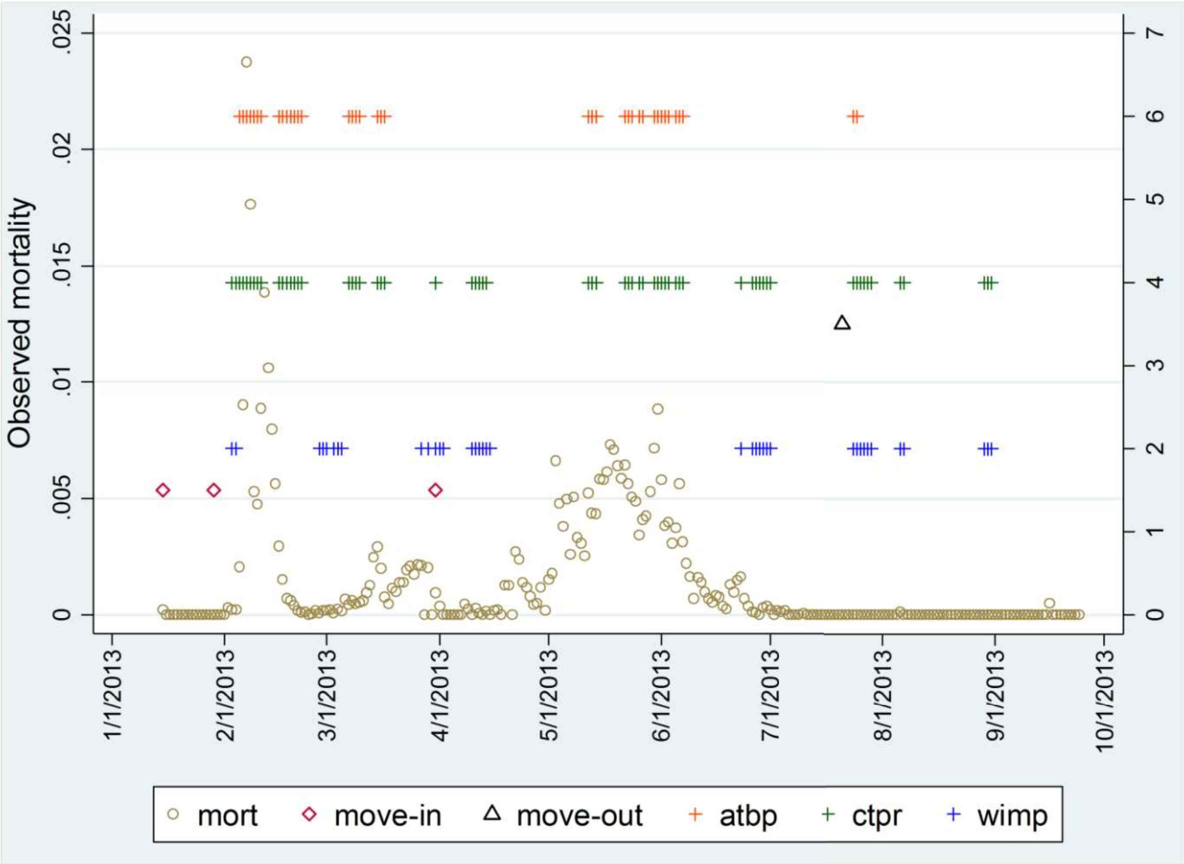

Observed mortality
0
.005
.01
.015
.02
.025
0
1
2
3
4
5
6
7
1/1/2013
2/1/2013
3/1/2013
4/1/2013
5/1/2013
6/1/2013
7/1/2013
8/1/2013
9/1/2013
10/1/2013
mort
move-in
move-out
atbp
ctpr
wimp

Fig.3.

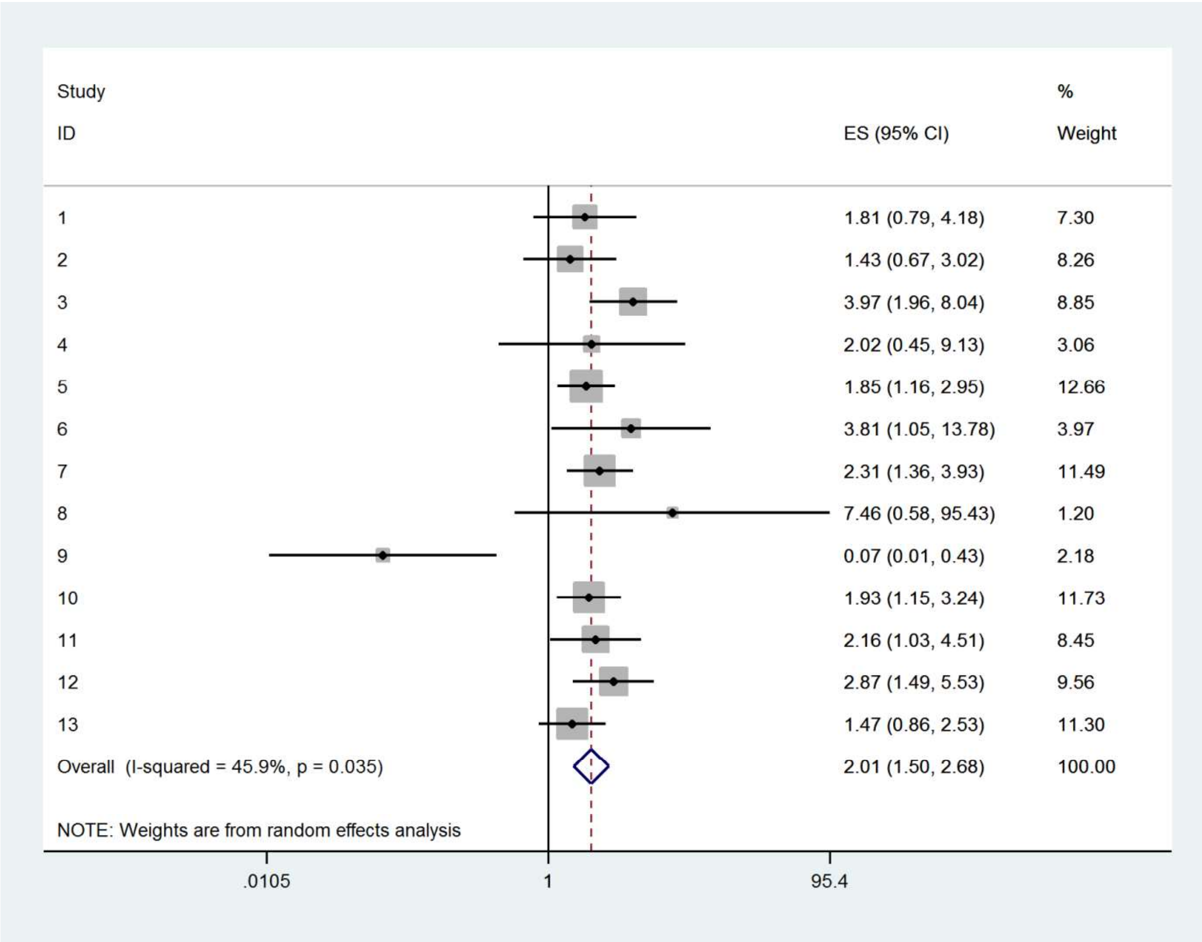
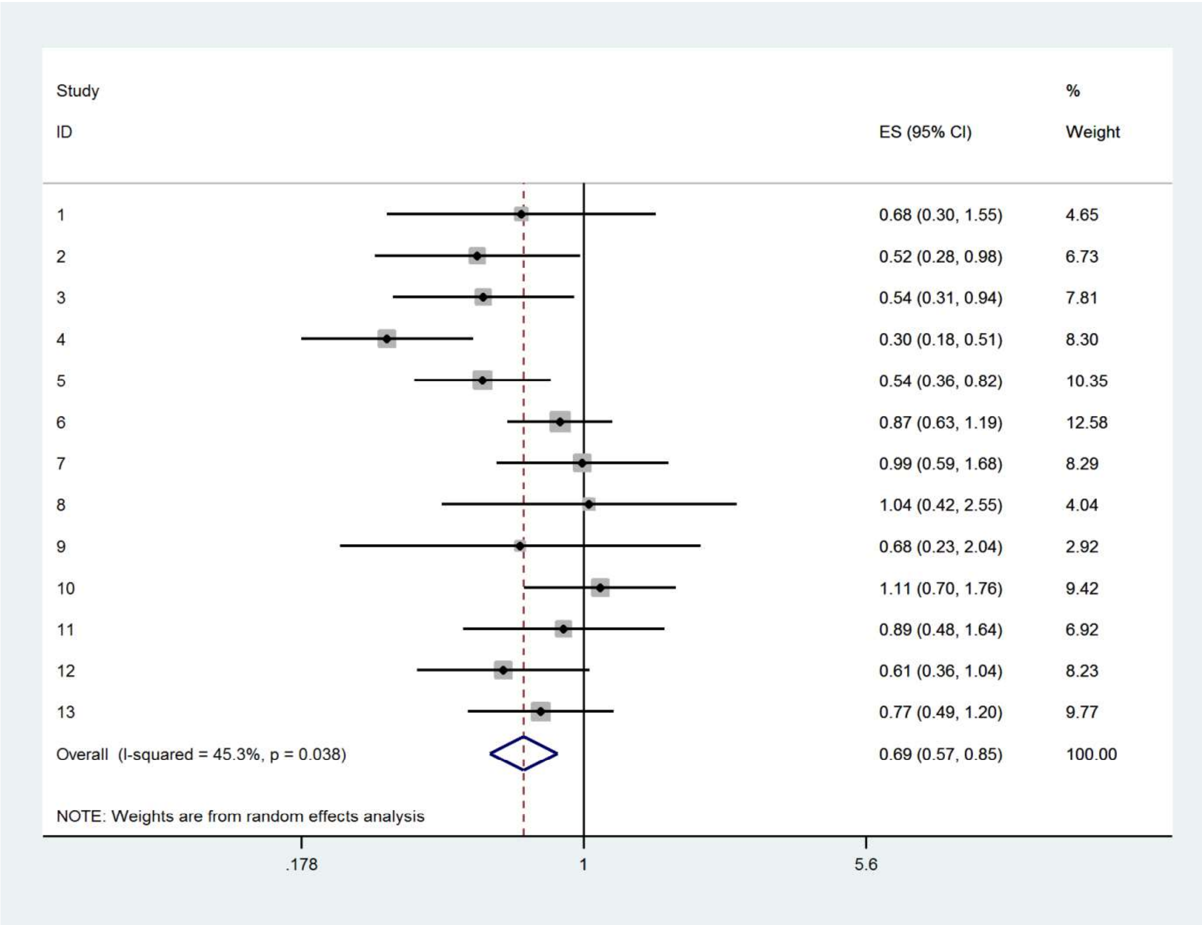

Study
ID
ES (95% CI)
%
Weight
1
1.81 (0.79, 4.18)
7.30
2
1.43 (0.67, 3.02)
8.26
3
3.97 (1.96, 8.04)
8.85
4
2.02 (0.45, 9.13)
3.06
5
1.85 (1.16, 2.95)
12.66
6
3.81 (1.05, 13.78)
3.97
7
2.31 (1.36, 3.93)
11.49
8
7.46 (0.58, 95.43)
1.20
9
0.07 (0.01, 0.43)
2.18
10
1.93 (1.15, 3.24)
11.73
11
2.16 (1.03, 4.51)
8.45
12
2.87 (1.49, 5.53)
9.56
13
1.47 (0.86, 2.53)
11.30
Overall (I-squared = 45.9%, p = 0.035)
2.01 (1.50, 2.68)
100.00
NOTE: Weights are from random effects analysis
.0105
1
95.4

Fig.4.


Study
ID
ES (95% CI)
%
Weight
1
0.68 (0.30, 1.55)
4.65
2
0.52 (0.28, 0.98)
6.73
3
0.54 (0.31, 0.94)
7.81
4
0.30 (0.18, 0.51)
8.30
5
0.54 (0.36, 0.82)
10.35
6
0.87 (0.63, 1.19)
12.58
7
0.99 (0.59, 1.68)
8.29
8
1.04 (0.42, 2.55)
4.04
9
0.68 (0.23, 2.04)
2.92
10
1.11 (0.70, 1.76)
9.42
11
0.89 (0.48, 1.64)
6.92
12
0.61 (0.36, 1.04)
8.23
13
0.77 (0.49, 1.20)
9.77
Overall (I-squared = 45.3%, p = 0.038)
0.69 (0.57, 0.85)
100.00
NOTE: Weights are from random effects analysis
.178
1
5.6

Supplementary materials for

A case study of time-series regression modeling: risk factors for pond-level mortality of farmed grass carp (*Ctenopharyngodon idella*) on a southern Chinese farm

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This file includes

S1. Exploratory descriptive analysis

S2. Main model selection

S3. Sensitivity analysis

S4. References

Tables S1-3

Figures S1-7

This document is presented as supplementary material for the manuscript submitted to Aquaculture.

S1. Other tests used in exploratory descriptive analysis

The sign test was applied to compare median mortality values of those matched-pair time windows of each pond. In order to compare the median differences in pond-level mortality before and after each intervention (e.g. movement and treatment of fish), we defined the following time windows for each management practice: (1) 3 days before and after movement-in of fish, movement-out of fish, and water improvement; (2) 7 days before and after treatment with antibiotics/antiparasitics and treatment with Chinese traditional medicine; and (3) 14 days before and after movement-in of fish. Based on sign tests carried out for each pond, there was almost no difference between the before-event mortalities and after-event mortalities, when each of the 6 management practices was individually evaluated for each time window (Tables S1a and 1b).

Generalized estimating equations (GEE) with an exchangeable correlation structure within ponds were used to test whether the mean mortality before exposure was equal to the mean after exposure, using data across all ponds. Because GEE were applied for marginal mean estimations with imbalanced clusters of fish mortalities in different ponds during the study period, we used an exchangeable correlation instead of independent, autoregressive, or unstructured correlation structures (Bergsma et al., 2009). All comparisons of GEE tests were not significant for both datasets with or without pond 33 ($P > 0.05$), indicating that after-intervention mortality was, in most cases, similar to before-intervention mortality (Table S2).

S2. Main model selection

Zero-inflated negative-binomial models with a constant zero-inflation proportion were compared to the negative-binomial models by Vuong's test (Hilbe, 2011). The zero-inflated models require a more complex estimation procedure and do not allow for deviance residuals. Hence, Pearson residuals (simple residuals divided by the standard deviation of observed counts) were used instead, although deviance residuals are generally preferable (Bhaskaran et al., 2013).

The Vuong test suggested an improvement in fit with a zero-inflated model over an ordinary negative-binomial model, for only two ponds (9 and 14) out of 13. A 5-knot spline was found to be the maximum number of knots for which negative-binomial models converged for all pond analyses. Including more knots caused the models to not converge for some ponds. Five knots has also been used in other TSR studies on mortality counts (Bhaskaran et al., 2013). In summary, we chose for our final model the following components: a negative binomial distribution (without zero-inflation), a 5-knot time spline, and two-lagged deviance residual terms. The robustness of our results with this model, in comparison with alternative model settings, was explored by a sensitivity analysis, as discussed in S3.

S3. Sensitivity analysis

In the first part of our sensitivity analysis, we compared the results of our selected model to 7 alternative models with slightly different features, as shown in Table S3. Two negative binomial models explored alternative ways of dealing with autocorrelation, by omitting the 2 deviance residual terms or by replacing them with a single lagged outcome term (settings 2-3). One negative-binomial model explored the impact of increasing the number of spline knots from 5 to 6, thereby excluding results from pond 23 (setting 4). Two zero-inflated negative-binomial models were explored, with either 5 or 6 spline knots and Pearson residual terms (settings 5-6), or replaced with a single lagged outcome term (setting 7). Finally, for the 5-spline knot model, with or without zero-inflation, estimation for each predictor on its own, instead of in a multivariable model with 7 predictors, was explored (settings 8-9).

The results of the sensitivity analysis are shown for each of the 7 predictors individually in Figures S1-7. For most predictors, the sensitivity analyses agreed on the direction, approximate confidence interval range, and overall significance (at $P<0.05$) of the coefficient. Exceptions were the univariable models for *mi3d* and *cpr7d*, the 2 models based on 6 spline knots for *atbp7d*, and the model unadjusted for autocorrelation for *tmax06*. These findings are discussed in the following paragraphs. Additionally, most I^2-values of different all-predictor models were within the range of 25-75%, indicating low to moderate levels of among-pond heterogeneity (Figs. S1-7).

The two predictors, *mi3d* and *mi2w,* had overlapping time intervals for the entry of fish because the 3 days of *mi3d* were also included in the 2-week interval of *mi2w*. In the univariable analysis, *mi3d* captured total mortality in the 3 days following movement, whereas in a multivariable model it captured additional mortality in those 3 days, relative to the general change in mortality during the 2 weeks after movement. The data showed that the 2-week effect was much stronger than the 3-day effect, explaining the difference between univariable and multivariable effects for mi3d and indicating that the former was the most relevant (Figs. S1 and 2).

The predictor *atbp7d* showed significant association in the 2 models with 6 spline knots, with the IRR estimates of 1.61 (setting 4) and 1.62 (setting 7), respectively, in contrast with the non-significant effect of *atbp7d* estimated by models with 5 spline knots (Fig. S4). This difference was essentially due to the exclusion of ponds 19 and 23 in the former models. In the 5-spline knot models without ponds 19 and 23, *atbp7d* was not significant ($P>0.05$), and its estimate was 1.36, which was different from IRRs estimated from all-variable models using 5 spline knots that ranged from 1.17-1.62 (Fig. S4). Because there was no objective reason to exclude ponds 19 and 23 from our analysis, we consider the results for the 5-spline knot model preferable.

The predictor *ctpr7d* was protective and significant in a multivariable model but showed no effect on its own (Fig. S5), and its inclusion strongly affected the coefficient for *ctpr7d*;

hence, the result of the multivariable analysis was the appropriate one to consider for *ctpr7d*. The different results can be explained as a confounding effect of temperature (*tmax06*), which was strongly associated with *ctpr7d* in some ponds where the treatments were confined to high temperature ranges.

The impact of *wimp3d* varied substantially across the sensitivity analyses, ranging in its estimated IRRs from 0.998 to 1.21, with the lowest estimates from the univariable analyses (Fig. S6). This appeared to be due less to a confounding effect of temperature (*tmax06*) than to a correlation with *ctpr7d*. Comparison of the group mean of *tmax06* indicated that water quality improvement was likely to happen on days with higher temperatures. Analyses with one or both of these predictors present showed that the overall significant conclusion for *ctpr7d* was not affected by the presence of wimp3d, while the reverse was not true. Additionally, among the multivariable analyses, both the number of spline knots and the distribution type appeared to impact the estimate to some degree. Because all changes in inference, relative to the final model, were towards the null, there may also be some selection bias from omitting ponds 19 and 23. A cautious conclusion would be that the results for the 5-spline knot model with all ponds are preferable. Considering these findings, we think it is fair to say that the results for *wimp3d* were inconclusive, but possibly suggestive of an increased risk.

There were some differences in estimates for *tmax06* across the models in our sensitivity analysis, although the range of estimates was relatively narrow, with IRRs from 1.11 to 1.19 (Fig. S7). This was not unexpected because this predictor was strongly time-varying, and model choices for time modeling (number of spline knots, adjustment for autocorrelation) would affect its estimate. The role of *tmax06* was to account for the biologically important impact of temperature and control for potential confounding effects on management factors of primary interest, so the differences in its estimate and standard error are not necessarily of concern.

Consistency of the 3 modeling components of the first-stage analysis was as follows:

(1) Distributional forms. We proposed a negative-binomial distribution as the main model for the among-pond analysis, and outcomes from different model options showed the robustness of our findings. Except for the univariable models, the estimated coefficients were fairly consistent for most predictors between the zero-inflated and negative-binomial models, after controlling for other modeling components. For modeling count data with excess zeros, distribution form would influence the standard errors more than the estimated associations (Lee et al., 2011; Imai et al., 2015). In our study, the estimated coefficients for *tmax06* and *ctpr7d* from the negative binomial and zero-inflated full models were generally consistent, but the confidence intervals varied slightly. However, this was not the case for the *mi2w* coefficient, for which the estimates and confidence interval were more similar when the estimation processes used the same combination of autocorrelation terms and spline functions under different distribution forms. In other studies, it might be worthwhile to explore whether the more elaborate model, i.e. zero-inflated model, would be helpful to improve model fit (Hilbe, 2011).

(2) Smooth function of time. The cubic spline used in this study is one of natural smoothing spline functions, which are useful to model non-linear association and capture autocorrelation in a TSR analysis (Armstrong, 2006). One needs to choose the number of knots as “a reasonable compromise between controlling for confounding bias by unmeasured risk factors changing smoothly over time (compromised by too few knots) and retaining enough exposure contrast from which to estimate an association (compromised by too many knots)” (personal comment by Ben Armstrong). Hence, the number of knots for this study (nk=5) might be

acknowledged as a reasonable choice. However, for one predictor (*atbp7d*), we found that models using 6 knots instead of 5 changed the estimates from non-significant to significant.

It is well-known that the number of knots (also called as the degrees of freedom of splines) and placement might influence the flexibility of fit and the estimated variances of the models (Katsouyanni et al., 2003; Bhaskaran et al., 2013). There are no uniform criteria to inform choices of the number of knots (Bhaskaran et al., 2013), and the decision could be data-driven or related to the specific data context targeted by the TSR method (Carder et al., 2005; Imai et al., 2015). It is still controversial whether the spline function can cause over-adjustment bias (Imai et al., 2015). In our study, the shift of the estimates of *atbp7d* could be due to either the model choices or the removal of the ponds 19 and 23 for the model with 6 knots. Compared with other estimates generated by the full models, interpretation of the association between mortality and water quality improvement might be less certain than those between mortality and of all other predictors. Furthermore, the model with 5 knots was able to avoid exclusion of ponds 19 and 23 data from the data analysis because of convergence problems that occurred when 6 knots were used.

(3) Autocorrelation. One of the autocorrelation terms used in this study was the log of the mortality count of the previous day (Peng et al., 2006; Imai et al., 2015), which is less commonly used than lagged residuals in TSR. However, it can be justified mathematically for infectious diseases, and might help with non-convergence (Imai and Hashizume, 2015). In our study, this autocorrelation approach was found to have a limited effect on the results.

S4. References

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Table S1 Nonparametric paired comparison between the median mortalities (x10^{-4}) of 3 or 14 days pre-movement and those of 3 or 14 days post-movement in each pond.

	3-day window of movement-in						14-day window of movement-in						3-day window of movement-out					
	Before		After		Sign test		Before		After		Sign test		Before		After		Sign test	
Pond	N	mort3d[b]	N	mort3d[a]	p1[a]	p2[b]	N	mort14d[b]	N	mort14d[a]	p1	p2	N	mort3d[b]	N	mort3d[a]	p1	p2
9	7	0	9	1.09	1	0.03	5	10.88	6	11.96	0.5	0.81	5	161.63	2	230.29	0.75	0.75
10	5	0	7	0	0.75	0.75	4	4.12	5	31.38	0.69	0.69	3	73.51	3	264.23	1	1
11	2	10.18	3	0.29	0.75	0.75	2	93.43	2	441.74	0.75	0.75	1	0	1	0	1	1
12	4	0	7	0	1	0.5	2	0.38	4	0.75	1	0.25	9	1.13	9	19.43	0.91	0.25
13	4	6.13	5	6.65	0.94	0.31	3	27.86	4	28.38	0.5	0.88	3	77.68	3	69.14	0.13	1
14	4	0	6	0	0.88	0.5	2	1.8	4	1.11	0.25	1	5	0	4	20.26	1	0.13
15	4	6.99	5	2.87	0.5	0.88	3	25.96	4	29.29	0.88	0.5	4	34.1	4	65.93	0.94	0.31
19	4	0	6	30.94	0.75	0.75	3	52.22	3	0	0.5	0.88	6	24.36	6	7.35	1	0.13
20	4	1.67	6	19.87	0.69	0.69	3	73.14	3	3.33	0.5	0.88	3	3.53	3	29	1	0.5
21	3	1.85	4	0.93	0.88	0.5	2	149.86	3	4.33	1	0.25	0					
22	3	3.84	4	1.28	0.5	0.88	1	93.32	3	3.84	1	0.5	1	66.42	1	86.22	1	0.5
23	3	2.05	4	1.36	0.75	0.75	2	24.62	3	1.37	1	0.5	0					
24	3	6.13	4	2.68	0.5	0.88	1	122.31	3	5.36	1	0.5	0					
33	6	0	8	0	1	0.5	5	0	5	0	0.88	0.5	1	509.76		0	1	1

Note: [a, b] One-sided sign test, with alternative hypotheses that probability of post-movement mortality was larger [a] (or smaller [b]) than pre-movement mortality, respectively. For example, if $p1<0.05$, the null hypothesis of equal probability of larger and smaller post-movement probability would be rejected in favour of a larger post-movement probability.

Table S1b Nonparametric paired comparison between the median mortalities ($x10^{-4}$) of 3 or 7 days pre-treatment and those of 3 or 7 days post-treatment in each pond.

	Antibiotics-antiparasitics						Chinese traditional medicine-probiotics						Water quality improvement					
	Before		After		Sign test		Before		After		Sign test		Before		After		Sign test	
Pond	N	mort7d[b]	N	mort7d[a]	p1[a]	p2[b]	N	mort7d[b]	N	mort7d[a]	p1	p2	N	mort3d[b]	N	mort3d[a]	p1	p2
9	17	141.83	17	341.72	0.99	0.02	37	15.29	37	14.25	0.95	0.09	35	8.74	35	6.59	0.7	0.43
10	10	189.07	10	1692.9	0.83	0.38	44	15.77	44	11.47	0.56	0.56	41	5.11	41	5.09	0.1	0.97
11	39	341.55	39	233.96	0.09	0.95	63	69.95	63	85.77	0.05	0.97	37	4.41	37	3.88	0.4	0.78
12	6	35.62	6	42.89	0.98	0.11	37	41.55	37	121.91	1	0	36	12.51	36	19.44	1	0.02
13	3	8.9	5	11.13	1	0.13	38	27.29	39	21.5	0.01	1	41	8.92	40	6.79	0.6	0.56
14	7	134.62	7	109.78	0.77	0.5	40	83.1	40	86.63	0.68	0.44	40	18.65	40	24.26	0.7	0.44
15	0		2	1.91	1	1	32	24.78	33	25.45	0.93	0.14	39	7.92	38	6.38	0.3	0.84
19	8	18.32	8	12.95	0.36	0.86	26	19.39	25	16.22	0.34	0.8	21	8.08	20	9.72	0.9	0.23
20	5	46.73	5	484.02	1	0.03	26	42.55	26	46.74	0.92	0.15	24	8.83	24	1.76	0.1	0.95
21	2	232.23	2	76.85	0.25	1	29	24.79	31	16.71	0.64	0.5	31	9.28	31	9.9	0.6	0.57
22	4	93.1	4	96.17	0.94	0.31	35	21.87	36	23.18	0.09	0.96	36	7.73	36	9.66	0.2	0.91
23	13	53.47	13	44.52	0.5	0.71	23	3.41	25	1.37	<0.01	1	34	11.58	34	9.58	0	0.99
24	4	52.14	4	79.81	0.69	0.69	39	59.26	40	40.71	0.05	0.97	40	23.05	40	14.2	0.2	0.9
33	13	1268.2	13	1164.1	0.5	0.71	35	6.12	35	0	0.41	0.75	40	0	40	0	0.4	0.77

Note: [a, b] One-sided sign test, with alternative hypotheses that probability of post-treatment mortality was larger [a] (or smaller [b]) than pre-treatment mortality, respectively. For example, if $p1<0.05$, the null hypothesis of equal probability of larger and smaller post-treatment probability would be rejected in favour of a larger post-treatment probability.

Table S2 Summary of generalized estimation equation results applied to the partial dataset with the pond 33 excluded when one of the following interventions took place.

Interventions and time window	**Estimated odds**[a]	**95% Confidence interval**	**P value**
3 days before and after movement-in of fish	0.86	(0.68, 1.10)	0.23
14 days before and after movement-in of fish	0.94	(0.61, 1.44)	0.77
3 days before and after movement-out of fish	1.98	(0.63, 6.25)	0.24
7 days before and after treatment with antibiotics or antiparasitics	1.48	(0.86, 2.56)	0.16
7 days before and after treatment with CTM or probiotics	0.93	(0.65, 1.33)	0.69
7 days before and after treatment with water improvement chemicals	0.86	(0.68, 1.10)	0.23

Note: [a] Odds referred to the probability of after-intervention mortality being larger than before-intervention mortality within the given time window divided by the probability of after-intervention mortality not being larger than before-intervention mortality within the given time window. CTM = traditional Chinese medicine.

Table S3 Sensitivity analyses. TSR models for full- and univariable- models substituted with different distributional forms, number of knots (nk) in spline, and autocorrelation options.

TSR Model abbreviation	Distributional form	Number of knots	Auto correlation term	Predictors included	Ponds analyzed
1. nb nk5 lag2 allvar	negative binomial	5	Deviance residual	All predictors	all 13 ponds[a]
2. nb nk5 noAC allvar	negative binomial	5	No residual	All predictors	all 13 ponds
3. nb nk5 logpre allvar	negative binomial	5	Logpregcdeath[b]	All predictors	all 13 ponds
4. nb nk6 lag2 allvar	negative binomial	6	Deviance residual	All predictors	all 13 ponds except pond 23
5. zinb nk5 lag2 allvar	zero-inflated negative binomial	5	Pearson residual	All predictors	all 13 ponds
6. zinb nk5 logpre allvar	zero-inflated negative binomial	5	Logpregcdeath	All predictors	all 13 ponds
7. zinb nk6 lag2 allvar	zero-inflated negative binomial	6	Pearson residual	All predictors	all 13 ponds except pond 23
8. nb nk5 lag2 univar	negative binomial	5	Deviance residual	Univariable	all 13 ponds
9. zinb nk5 lag2 univar	zero-inflated negative binomial	5	Pearson residual	Univariable	all 13 ponds

Note: [a] Among the originally recorded 14 ponds, all the other 13ponds were included in the time series analysis except pond 33.

[b] Logpregcdeath denoted as the previous day logarithmic transformed count of mortalities.

Figure legends (Figs. S1-7)

Fig. S1. Sensitivity analysis for estimation of incidence rate ratio (IRR) of movement-in of fish in the previous 3 days (*mi3d*) using all-predictor and univariable models.

Fig. S2. Sensitivity analysis for estimation of incidence rate ratio (IRR) of movement-in of fish in the previous 2 weeks (*mi2w*) using all-predictor and univariable models.

Fig. S3. Sensitivity analysis for estimation of incidence rate ratio (IRR) of movement-in of fish in the previous 3days (*mo3dm*) using all-predictor and univariable models.

Fig. S4. Sensitivity analysis for estimation of incidence rate ratio (IRR) of treatment with antibiotics or antiparasitics during the previous 7 days (*atpbp7d*) using all-predictor and univariable models.

Fig. S5. Sensitivity analysis for estimation of incidence rate ratio (IRR) of treatment with CTM or probiotics during the previous 7 days (*ctpr7d*) using all-predictor and univariable models.

Fig. S6. Sensitivity analysis for estimation of incidence rate ratio (IRR) of water quality treatment during the previous 3 days (*wimp3d*) using all-predictor and univariable models.

Fig. S7. Sensitivity analysis for estimation of incidence rate ratio (IRR) of temperature of previous week increase by 1^{0}C (*tmax06)* using all-predictor and univariable models.

Fig. S1.

Model	P	IRR	95% CI		Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.46	0.83	(0.52,	1.35)	0.003	59.8%
2. nb nk5 noAC allvar	0.25	0.79	(0.52,	1.18)	0.050	42.9%
3. nb nk5 logpre allvar	0.68	0.99	(0.57,	1.72)	<0.001	71.0%
4. nb nk6 lag2 allvar	0.47	0.83	(0.51,	1.37)	0.013	55.2%
5. zinb nk5 lag2 allvar	0.36	0.79	(0.48,	1.30)	<0.001	67.8%
6. zinb nk5 logpre allvar	0.83	1.07	(0.58,	1.98)	<0.001	79.6%
7. zinb nk6 lag2 all var	0.43	0.80	(0.46,	1.39)	<0.001	68.7%
8. nb nk5 lag2 univar	0.16	1.53	(0.84,	2.78)	<0.001	79.6%
9. zinb nk5 lag2 univar	0.12	1.48	(0.90,	2.44)	<0.001	72.5%

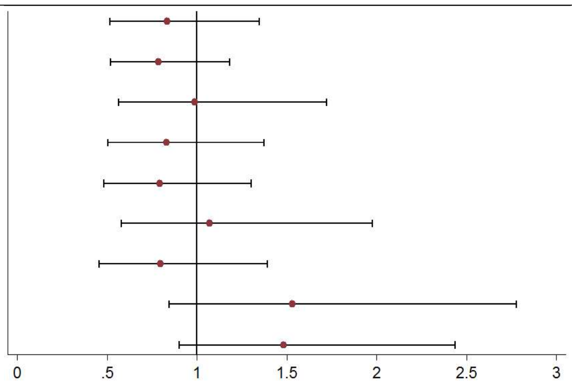


Fig. S2.

Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	<0.001	2.01	(1.50, 2.67)	0.04	45.9%
2. nb nk5 noAC allvar	<0.001	2.00	(1.55, 2.59)	0.15	29.3%
3. nb nk5 logpre allvar	0.001	1.63	(1.24, 2.16)	0.07	39.6%
4. nb nk6 lag2 allvar	<0.001	1.87	(1.36, 2.58)	0.03	49.4%
5. zinb nk5 lag2 allvar	<0.001	1.97	(1.65, 2.35)	0.49	0
6. zinb nk5 logpre allvar	0.003	1.55	(1.16, 2.05)	0.02	49.6%
7. zinb nk6 lag2 all var	<0.001	1.96	(1.42, 2.70)	0.01	56.7%
8. nb nk5 lag2 univar	<0.001	2.24	(1.49, 3.37)	<0.001	76.9%
9. zinb nk5 lag2 univar	<0.001	2.54	(1.66, 3.89)	<0.001	81.6%

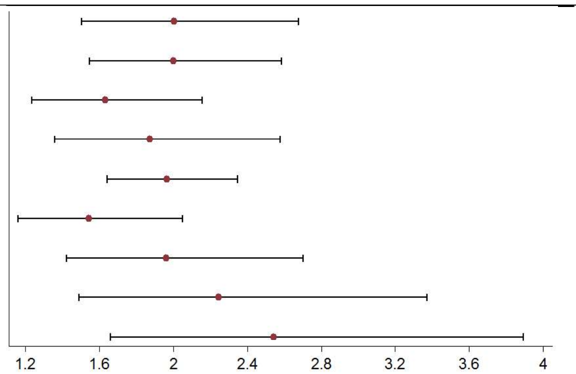


Fig. S3.

Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.22	1.37	(0.83, 2.26)	0.05	48.70%
2. nb nk5 noAC allvar	0.15	1.37	(0.90, 2.09)	0.21	26.00%
3. nb nk5 logpre allvar	0.08	1.45	(0.96, 2.18)	0.21	25.90%
4. nb nk6 lag2 allvar	0.26	1.34	(0.81, 2.23)	0.11	40.70%
5. zinb nk5 lag2 allvar	0.20	1.42	(0.84, 2.42)	0.02	56.90%
6. zinb nk5 logpre allvar	0.08	1.46	(0.96, 2.23)	0.15	34.00%
7. zinb nk6 lag2 all var	0.27	1.37	(0.78, 2.42)	0.03	55.90%
8. nb nk5 lag2 univar	0.16	1.46	(0.87, 2.45)	0.05	48.80%
9. zinb nk5 lag2 univar	0.14	1.53	(0.87, 2.67)	0.01	58.70%

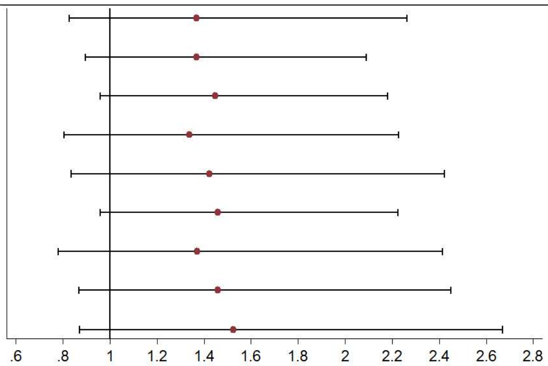


Fig. S4.

Model	P	IRR	95% CI		Heterogeneity P	I²
1. nb nk5 lag2 allvar	0.08	1.28	(0.97,	1.69)	0.04	46.00%
2. nb nk5 noAC allvar	0.07	1.26	(0.98,	1.61)	0.16	28.30%
3. nb nk5 logpre allvar	0.21	1.17	(0.92,	1.50)	0.13	32.00%
4. nb nk6 lag2 allvar	0.003	1.61	(1.18,	2.20)	0.04	47.30%
5. zinb nk5 lag2 allvar	0.07	1.34	(0.97,	1.85)	< 0.001	70.00%
6. zinb nk5 logpre allvar	0.17	1.20	(0.93,	1.56)	0.01	53.30%
7. zinb nk6 lag2 all var	0.02	1.62	(1.10,	2.39)	< 0.001	74.90%
8. nb nk5 lag2 univar	0.69	1.08	(0.73,	1.61)	< 0.001	82.80%
9. zinb nk5 lag2 univar	0.73	1.07	(0.73,	1.59)	< 0.001	86.70%

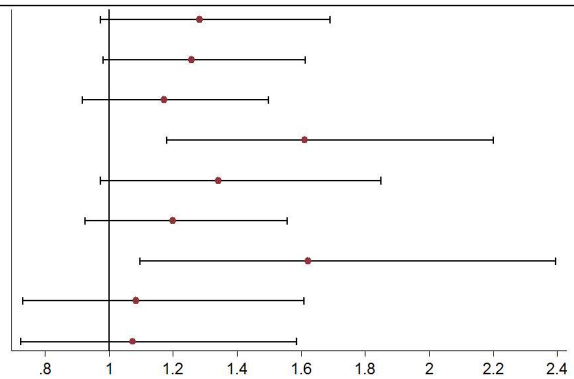
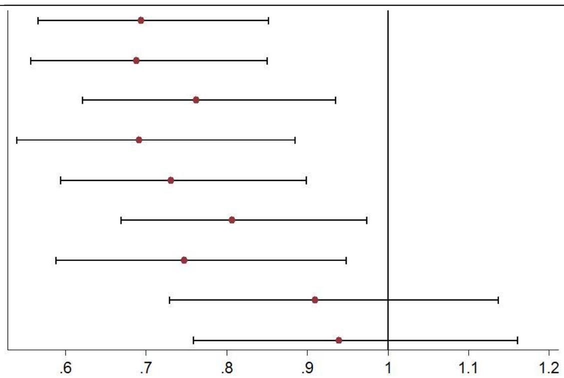
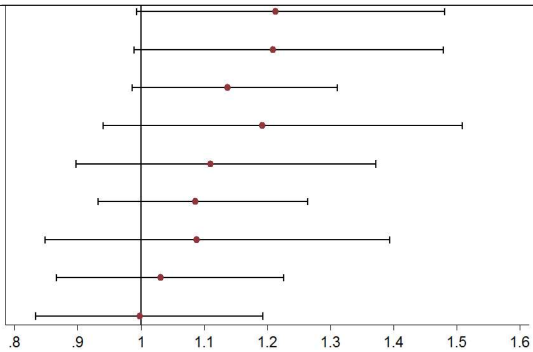


Fig. S5.



Model	P	IRR	95% CI		Heterogeneity P	I²
1. nb nk5 lag2 allvar	< 0.001	0.69	(0.57,	0.85)	0.04	45.3%
2. nb nk5 noAC allvar	0.001	0.69	(0.56,	0.85)	0.04	44.0%
3. nb nk5 logpre allvar	0.01	0.76	(0.62,	0.94)	0.04	45.0%
4. nb nk6 lag2 allvar	0.003	0.69	(0.54,	0.89)	0.01	57.3%
5. zinb nk5 lag2 allvar	0.003	0.73	(0.59,	0.90)	0.01	56.8%
6. zinb nk5 logpre allvar	0.03	0.81	(0.67,	0.97)	0.03	47.6%
7. zinb nk6 lag2 all var	0.02	0.75	(0.59,	0.95)	0.002	63.5%
8. nb nk5 lag2 univar	0.42	0.91	(0.73,	1.14)	< 0.001	74.6%
9. zinb nk5 lag2 univar	0.56	0.94	(0.76,	1.16)	< 0.001	78.1%

Fig. S6.



Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.06	1.21	(0.99, 1.48)	0.04	45.80%
2. nb nk5 noAC allvar	0.06	1.21	(0.99, 1.48)	0.06	40.80%
3. nb nk5 logpre allvar	0.08	1.14	(0.99, 1.31)	0.44	0.10%
4. nb nk6 lag2 allvar	0.15	1.19	(0.94, 1.51)	0.01	57.00%
5. zinb nk5 lag2 allvar	0.34	1.11	(0.90, 1.37)	0.001	63.30%
6. zinb nk5 logpre allvar	0.29	1.09	(0.93, 1.26)	0.16	28.30%
7. zinb nk6 lag2 all var	0.50	1.09	(0.85, 1.39)	< 0.001	70.60%
8. nb nk5 lag2 univar	0.73	1.03	(0.87, 1.23)	0.01	56.70%
9. zinb nk5 lag2 univar	0.98	1.00	(0.83, 1.19)	< 0.001	68.10%

Fig. S7.

Model	P	IRR	95% CI	Heterogeneity P	I^2
1. nb nk5 lag2 allvar	0.001	1.17	(1.06, 1.28)	<0.001	78.7%
2. nb nk5 noAC allvar	0.001	1.17	(1.07, 1.28)	<0.001	76.3%
3. nb nk5 logpre allvar	< 0.001	1.12	(1.05, 1.19)	0.02	49.7%
4. nb nk6 lag2 allvar	0.003	1.16	(1.05, 1.27)	<0.001	75.7%
5. zinb nk5 lag2 allvar	< 0.001	1.16	(1.07, 1.25)	<0.001	76.8%
6. zinb nk5 logpre allvar	0.002	1.11	(1.04, 1.18)	0.002	60.6%
7. zinb nk6 lag2 all var	0.001	1.17	(1.07, 1.27)	<0.001	77.2%
8. nb nk5 lag2 univar	0.002	1.18	(1.06, 1.31)	<0.001	86.0%
9. zinb nk5 lag2 univar	0.001	1.19	(1.08, 1.32)	<0.001	88.0%