Electronic Material

Spatial epidemiological modelling of infection by *Vibrio aestuarianus* shows that connectivity and temperature control oyster mortality

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Text S1. Details of epidemiological parameter values at low seawater temperatures observed in the experimental infection trials

Dedicated experimental trials were designed to measure the epidemiological parameter values at different seawater temperatures, as previously described by Lupo et al. 2019. Seawater temperature values were roughly chosen based on the temperature monitoring of the Baie des Veys coastal waters (REPHY, 2017).

Briefly, two years old hatchery-produced oysters were kept in experimental facilities with UV-treated and filtered seawater and under biosecurity conditions to avoid contamination with naturally occurring pathogens. They were acclimated at desired temperature (i.e. 10 or 15°C) following a 2°C-day. Batches of adult oysters were infected with *Vibrio aestuarianus* 02/041-GFP-tagged by intramuscular injection or immersion into contaminated seawater. Oysters (10 per temperature condition) were transiently exposed to the contaminated seawater by immersion in individual beakers for 24h at 10 and 15°C, respectively. Then, they were transferred in individual clean beakers, under static (closed circuit) conditions in aerated seawater maintained at desired temperatures during all experiments. The following observations were daily recorded:

(1) Oysters were monitored daily and dead or moribund animals were removed and frozen for subsequently sampling for the duration of the experiment (10 days post exposure). Survivors were sacrificed at the end of the experiment. Individual mantle and gills samples, or whole animals in case of survivors, were tested for the presence of *V. aestuarianus* DNA.

(2) Bacterial shedding period and shedding concentration were estimated by a daily monitoring of GFP-bacteria in seawater. Briefly, oysters were daily transferred in new beakers containing fresh but acclimated UV-treated seawater. Each 24h during 10 days, surrounding seawater was sampled and analysed, and oysters were transferred into a new beaker.

(3) Free-living-bacteria liefetime was estimated by following GFP-bacteria concentration in different seawater samples placed at different temperatures. Briefly, seawater samples from different beakers containing excreting oysters were divided in two equivalent parts and placed in incubators with a constant agitation. During 7 days, GFP-bacteria concentration was daily measured by flow cytometry.

Four temporal individual trials were conducted (2 dose conditions x 2 trials x 10 oysters) in this study, allowing two replicates of the transmission trials for both the 10 and 15°C conditions.

Table S1: Values of the model parameters at the different seawater temperatures

Parameter	Unit	10°C						15°C					
		N	Min	Q1	Median	Q3	Max	N	Min	Q1	Median	Q3	Max
Latency period ($1/\rho$)	Days^{-1}	1	11	11	11	11	11	4	8	8.75	9.5	10.75	13
Infectious period ($1/r$)	Days^{-1}	16	1	3.75	7	9.25	11	22	0	2	3	4	8
Bacteria shedding rate (e)	Bacteria/mL per day per oyster	75	1,080	5,060	23,500	223,000	1,850,000	82	1,110	26,150	98,450	371,000	2,580,000
Free-living–bacteria lifetime in seawater ($1/\xi$)	Days	20	4	4	4	5	5	20	3	3	3	4	5

Text S2. Theoretical R_0 calculation

The Next Generation Matrix (NGM) method has been applied (Diekmann et al. 2010) to derive the theoretical R_0 calculation.

The ordinary differential equations (ODE) are:

$$\frac{dS}{dt} = -a \cdot \lambda(P) \cdot S \qquad (S1)$$
$$\frac{dE}{dt} = a \cdot \lambda(P) \cdot S - \rho \cdot E \qquad (S2)$$
$$\frac{dI}{dt} = \rho \cdot E - r \cdot I \qquad (S3)$$
$$\frac{dW}{dt} = e \cdot I - \xi \cdot P \qquad (S4)$$

with:

$$\lambda(P) = \frac{P}{K+P}, \qquad (S5)$$

with $N = S + E + I$. This system has 3 infected classes, exposed oysters (E), infected oysters (I) and free-living bacteria in the seawater (P).

At the disease-free equilibrium (DFE), the disease has disappeared from the system or has not yet entered it, *i.e.* there is no infected or infectious hosts (E=0 and I=0) and there is no free-living bacteria in the seawater (P=0). The number of oysters is $S=S_0$.

The matrix **T** corresponds to transmissions if E_0=1 or I_0=1 or if P_0 bacteria are introduced into the system at the DFE. It includes all epidemiological events that lead to new infections. The matrix **Σ** corresponds to transitions among states, and includes all other events, *i.e.* all exits from the infected classes and all entries into the infected classes for other reasons than the generation of a new infected entity (infected animal or free-living bacteria).

In our system, we have 3 infected classes thus the matrices **T** and **Σ** are three-dimensional [equations (S6) and (S7)]. They are obtained from equations (S2), (S3) and (S4) by separating the transmission events from other events. In the matrices, the upper left term corresponds to the partial derivative of the differential equation dE/dt with respect to E, considering I and P constant. The upper middle term corresponds to the partial derivative of the differential equation dE/dt with respect to I, considering E and P constant, and so on for other terms of the matrix. Especially, the only new infected entities generated are either shed bacteria (equal to *e* if one infectious animal is introduced at DFE), or newly infected animals (of state E, first line third column of matrix **T**) if seawater is initially contaminated at level P_0. However, in our simulations, the initialization of the system is based on the introduction of infected animals only (of state I) thus P_0=0. The number of bacteria shed by the initially infected animals is small, thus the concentration of bacteria in the seawater P_0 doesn't reach K quickly during the first time step. As a consequence, P_0 is discarded in front of K.

$$\mathbf{T} = \begin{pmatrix} 0 & 0 & \frac{aS_0}{K} \\ 0 & 0 & 0 \\ 0 & e & 0 \end{pmatrix} \quad (S6)$$

$$\boldsymbol{\Sigma} = \begin{pmatrix} -\rho & 0 & 0 \\ \rho & -r & 0 \\ 0 & 0 & -\xi \end{pmatrix} \quad (S7)$$

The NGM is defined as $\mathbf{K} = -\mathbf{T}.\boldsymbol{\Sigma}^{-1}$

To find the inverse of a three-dimensional matrix $A = \begin{pmatrix} a & b & c \\ d & e & f \\ g & h & i \end{pmatrix}$, first we find the determinant (det) of matrix A:

$\det(A) = aei + dhc + gbf - ahf - dbi - gec$

Second, we find the transpose of the cofactor matrix C:

$$C = \begin{pmatrix} +\begin{vmatrix} e & f \\ h & i \end{vmatrix} & -\begin{vmatrix} d & f \\ g & i \end{vmatrix} & +\begin{vmatrix} d & e \\ g & h \end{vmatrix} \\ -\begin{vmatrix} b & c \\ h & i \end{vmatrix} & +\begin{vmatrix} a & c \\ g & i \end{vmatrix} & -\begin{vmatrix} a & b \\ g & h \end{vmatrix} \\ +\begin{vmatrix} b & c \\ e & f \end{vmatrix} & -\begin{vmatrix} a & c \\ d & f \end{vmatrix} & +\begin{vmatrix} a & b \\ d & e \end{vmatrix} \end{pmatrix} = \begin{pmatrix} ei - hf & fg - di & dh - ge \\ ch - bi & ai - gc & gb - ah \\ bf - ec & dc - af & ae - db \end{pmatrix}$$

And $C^T = \begin{pmatrix} ei - hf & ch - bi & bf - ec \\ fg - di & ai - gc & dc - af \\ dh - ge & gb - ah & ae - db \end{pmatrix}$

$A^{-1} = \frac{1}{\det(A)} \times C^T$

We have: $\det(\boldsymbol{\Sigma}) = (-\rho \times -r \times -\xi) + (\rho \times 0 \times 0) + (0 \times 0 \times 0) - (-\rho \times 0 \times 0) - (\rho \times 0 \times -\xi) - (0 \times -r \times 0) = -\rho r \xi$

The determinant of matrix $\boldsymbol{\Sigma}$ is not null, thus $\boldsymbol{\Sigma}$ can be inversed.

Then $\boldsymbol{\Sigma}^{-1} = \frac{1}{-\rho r \xi} \times \begin{pmatrix} r\xi & 0 & 0 \\ \rho\xi & \rho\xi & 0 \\ 0 & 0 & \rho r \end{pmatrix} = \begin{pmatrix} -\frac{1}{\rho} & 0 & 0 \\ -\frac{1}{r} & -\frac{1}{r} & 0 \\ 0 & 0 & -\frac{1}{\xi} \end{pmatrix} \quad (S8)$

Thus $\mathbf{K} = -\mathbf{T}.\mathbf{\Sigma}^{-1} = \begin{pmatrix} 0 & 0 & \frac{aS_0}{K} \\ 0 & 0 & 0 \\ 0 & e & 0 \end{pmatrix} \times \begin{pmatrix} \frac{1}{\rho} & 0 & 0 \\ \frac{1}{r} & \frac{1}{r} & 0 \\ 0 & 0 & \frac{1}{\xi} \end{pmatrix} = \begin{pmatrix} 0 & 0 & \frac{aS_0}{\xi K} \\ 0 & 0 & 0 \\ \frac{e}{r} & \frac{e}{r} & 0 \end{pmatrix}$ (S9)

Then, to find the eigenvalues of $\mathbf{K}$, let solve $\det(-\mathbf{T}.\mathbf{\Sigma}^{-1} - \lambda I) = 0$, I being the identity matrix.

$B = -\mathbf{T}.\mathbf{\Sigma}^{-1} - \lambda I = \begin{pmatrix} 0 & 0 & \frac{aS_0}{\xi K} \\ 0 & 0 & 0 \\ \frac{e}{r} & \frac{e}{r} & 0 \end{pmatrix} - \begin{pmatrix} \lambda & 0 & 0 \\ 0 & \lambda & 0 \\ 0 & 0 & \lambda \end{pmatrix} = \begin{pmatrix} -\lambda & 0 & \frac{aS_0}{\xi K} \\ 0 & -\lambda & 0 \\ \frac{e}{r} & \frac{e}{r} & -\lambda \end{pmatrix}$ (S10)

$\text{Det}(B) = -\lambda^3 + \frac{e\lambda aS_0}{r\xi K}$ (S11)

$\text{Det}(B) = 0 \Leftrightarrow -\lambda^3 + \frac{e\lambda aKS_0}{r\xi K} = 0 \Leftrightarrow \lambda = 0 \text{ or } \lambda^2 = \frac{eaS_0}{r\xi K}$

The largest eigenvalue corresponds to R_0.

$R_0 = \sqrt{\frac{eaS_0}{r\xi K}}$ (S12)

1. References

Diekmann O, Heesterbeek JA, Roberts MG (2010) The construction of next-generation matrices for compartmental epidemic models. Journal of the Royal Society, Interface / the Royal Society 7:873-885

Lupo C, Travers M-A, Tourbiez D, Barthélémy CF, Beaunée G, Ezanno P (2019) Modeling the transmission of *Vibrio aestuarianus* among Pacific oysters using experimental infection data. Front Vet Sci 6:142