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Kerry A. Hamilton[a,*], Arlene Chen[b], Emmanuel de-Graft Johnson[c], Anna Gitter[d], Sonya Kozak[e], Celma Niquice[f], Amity G. Zimmer-Faust[g], Mark H. Weir[h], Jade Mitchell[i], Patrick L. Gurian[a]

[a] Department of Civil, Architectural, and Environmental Engineering, Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA
[b] Maryland Pathogen Research Institute, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA
[c] Department of Mathematics, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology, SCB/AMC SF 24/B6-KNUST, Kumasi, Ghana
[d] Water Management and Hydrological Sciences Program, Texas A&M University, 400 Bizzell Street, College Station, TX 77843, USA
[e] School of Medicine, Griffith University, Gold Coast, Australia
[f] Faculty of Civil Engineering and Geosciences, Technical University of Delft, Netherlands
[g] Western Ecology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, USA
[h] Division of Environmental Health Sciences and Department of Civil Environmental and Geodetic Engineering, The Ohio State University, USA
[i] Department of Biosystems and Agricultural Engineering, Michigan State University, 524 S. Shaw Lane, East Lansing, MI 48824, USA

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ABSTRACT

The use of aquaculture is increasing to meet the growing global demand for seafood. However, the use of aquaculture for seafood production incurs potential human health risks, especially from enteric bacteria such as *Salmonella* spp. *Salmonella* spp. was the most frequently reported cause of outbreaks associated with crustaceans from 1998 to 2004. Among crustacean species, shrimp are the most economically important, internationally traded seafood commodity, and the most commonly aquaculture-raised seafood imported to the United States. To inform safe aquaculture practices, a quantitative microbial risk assessment (QMRA) was performed, incorporating stochastic variability in pathogen growth, industrial shrimp processing, and consumer shrimp preparation. Several scenarios including gamma irradiation and cooking time were considered in order to examine the relative importance of these practices in terms of their impact on risk. Median annual infection risks for all scenarios considered were below 10^{-4} and median disability adjusted life year (DALY) metrics were below 10^{-6} DALY per person per year, however, 95th percentile risks were above 10^{-4} annual probability of infection and 10^{-6} DALY per person per year for scenarios with improper cooking and lack of gamma irradiation. The greatest difference between microbiological risks for the scenarios tested was observed when comparing proper vs. improper cooking (5–6 orders of magnitude) and gamma irradiation (4–5 orders of magnitude) compared to (up to less than 1 order of magnitude) for peeling and "deveining" (removing the shrimp digestive tract) vs. peeling only. The findings from this research suggest that restriction of *Salmonella* spp. to low levels (median 5–30 per L aquaculture pond water) may be necessary for scenarios in which proper downstream food handling and processing cannot be guaranteed.

1. Introduction

With the increase in global population and seafood consumption, aquaculture practices are essential for meeting global seafood demands. Aquaculture supplied 44% of all animal seafood to consumers in 2014, and is projected to surpasse production from capture fisheries in 2021 (FAO, 2016). The United States continues to be a primary consumer of aquaculture, with around 91% of total seafood consumed originating abroad, causing a seafood trade deficit of over $11.2 billion per year (NOAA, 2017).

Shrimp have been identified as the most economically important, internationally traded seafood commodity (Amagliani et al., 2012). Moreover, penaeid shrimps are the most commercially important species of farmed shrimp globally (FAO, 2016; Farfante, 1988; Moss et al., 2012). *Penaeus vannamei*, whiteleg shrimp, have been effectively grown in multiple states in the US, including Alabama, Florida, Hawaii, Nevada, Michigan, Indiana, Iowa, Maryland, Massachusetts, and the US territory of Guam (Treece, 2014). Shrimp is also the most common aquaculture-raised seafood imported to the United States, followed by Atlantic salmon, tilapia, and shellfish with Asian countries and Ecuador supplying the majority of imported shrimp (NOAA, 2017). In addition, shrimp is the most commonly consumed seafood product in terms of

* Corresponding author.
E-mail address: kh495@drexel.edu (K.A. Hamilton).

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annual consumption; on average, 4.10 pounds per person per year are consumed in the US (National Advisory Committee on Microbiological Criteria for Foods, 2008).

With increasing population growth and the need for sustainable aquaculture practices, the human health concerns for the consumption of aquaculture products grown in water of variable quality should be reviewed as chemical (including hormones), biological, heavy metals, and emerging contaminants such as antibiotic resistant bacteria have been identified as potential public health risks (Gormaz et al., 2014; Sapkota et al., 2008). In some cases, wastewater-fed aquaculture can be practiced (Prein, 1990; Stenström et al., 2011; Strauss, 1996; Strauss and Blumenthal, 1990). Although this is not currently practiced for some high-value species such as shrimp due to the need for strict control of ammonia (Alcaraz et al., 1999; WHO, 2006), wastewater-fed aquaculture may be practiced with a combination of cultured seafood products (including shrimp) in a single system (Strauss and Blumenthal, 1990), or could be used to culture fish or aquatic plants used as animal feed for other high-value fish and shrimp (United Nations Environment Programme, 2000). In the future, such practice might be considered for shrimp using waste stabilization ponds with long detention times or using tertiary treated wastewater. As seafood demand increases, a variety of water sources will likely be considered for aquaculture and as such, guidance would be useful with regard to appropriate water quality targets.

The World Health Organization uses a harmonized approach to risk assessment and management for aquaculture consistent with the Stockholm Framework, which involves “the assessment of health risks prior to the setting of health-based targets and the development of guideline values, defining basic control approaches and evaluating the impact of these combined approaches on public health” (WHO, 2006). While metals and chemicals can incur potentially meaningful, long-term health risks in the context of aquaculture, the driving human health risk concern is exposure to pathogenic microorganisms (Strauss 1996). Within the Stockholm Framework, the assessment of health risks can be performed using epidemiological studies, or quantitative microbial risk assessment (QMRA). Due to the challenges of assessing environmental health risks based on epidemiologic data due to the need for large studies in order to detect small changes in a given health outcome, QMRAs are increasingly used to inform engineering and public health decisions related to food and water (Ashbolt et al., 2010; Hathaway and Cook, 1997).

QMRA follows a four step process – hazard identification, exposure assessment, dose-response assessment, and risk characterization (Haas et al., 2014). This process is especially useful for characterizing risks at low doses of pathogens that might be difficult to do with an epidemiologic study. During hazard identification, the primary pathogen of concern is identified and the transmission routes and disease outcomes are described in order to frame the problem statement. Exposure assessment characterizes the occurrence of pathogens in the environment, and models their fate and transport up to the point of arriving at the target organ of a human receptor. The dose response analysis provides a mathematical relationship for the dose arriving at the target organ and a probability of a given health endpoint. The risk characterization process contextualizes this information into a risk estimate by accounting for exposure frequencies and durations.

To date, only a few quantitative risk assessments have been performed for seafood consumption in order to inform risk management practices (Chanpiwat et al., 2016; Iwahori et al., 2010; Rico and Van den Brink, 2014; Sani et al., 2013; Yajima and Kurokura, 2008). Other types of risk assessments have been performed for aquaculture with regards to health impact assessment (Winkler et al., 2017a), qualitative risk matrices (Stenström et al., 2011), or best-management practices (Winkler et al., 2017b). Although these approaches are valuable, QMRA provides the best opportunity to quantify the impact of different scenarios and identify water quality target ranges. Limited microbiological water quality guidance is available for identifying these target ranges for aquaculture, and existing guidance values are not risk-based. Recommended microbial quality targets for aquaculture to protect consumers are $\leq 10^4$ *E. coli* per 100 mL for pond water; target values are up to one order of magnitude lower (10^3–10^4 per 100 mL) for agricultural workers and local communities, respectively (WHO, 2006). The US Environmental Protection Agency regulates discharges from aquaculture ponds through the National Pollutant Discharge Elimination System (NPDES) (40 CFR Part 451), but does not regulate the quality of water in the ponds themselves. There are no other US requirements for pond microbiological water quality, however, the FDA recommends the use of a hazard analysis and critical control point (HACCP) approach for aquaculture facilities (USFDA, 2008).

While viruses may be a good indicator of fecal contamination and result in higher predicted risks due to their low median infectious doses compared to many bacteria (Haas et al., 2014), most common seafood inspection analyses would not include viruses due to limitations regarding the ability to culture them in routine practice, for example a standardized culture method is not yet available for norovirus. Management and/or inspection practices would most likely focus on monitoring common bacterial pathogens such as *Listeria* spp. and *Salmonella* spp. (USFDA, 2008). Furthermore, the cooking guidance in the 2005 FDA Food Code used *Salmonella* spp. as a target pathogen for their cooking time and temperature recommendations for food products including seafood (National Advisory Committee on Microbiological Criteria for Foods, 2008; USFDA, 2005). Apart from HACCP critical control points, the USFDA policy regarding adulteration of seafood is that it cannot contain *Salmonella* spp. (USFDA, 1999). While the Food and Drug Administration (FDA) requires inspection of the safety of shrimp products, only 0.7% of shrimp imports were reported to be inspected and over 58% of total seafood products identified as being contaminated with *Salmonella* spp. were shrimps and prawns (Consumer Reports, 2015).

Salmonella spp. is one of the leading causes of foodborne illnesses in the United States, contributing to at least 1.4 million cases annually (Iwamoto et al., 2010). The most common causal agents of outbreaks in shellfish associated with fecal pollution in the US from 1998 to 2004 were *Salmonella* spp. (1119 *Salmonella* spp. cases of 7685 total foodborne illness cases associated with seafood) and norovirus (1533 norovirus cases of the 7685 total cases) (National Advisory Committee on Microbiological Criteria for Foods, 2008). *Salmonella* spp. was furthermore the most frequently reported cause of US foodborne illness outbreaks associated with crustaceans in the US from 1998 to 2004, with 10 (and another 3 suspected) outbreaks associated with consumption of shrimp contaminated with *Salmonella* spp. over this period (National Advisory Committee on Microbiological Criteria for Foods, 2008; Norhana et al., 2010). Salmonellosis has been linked to consumption of shrimp grown in aquaculture ponds (Koonse et al., 2005) and is of great concern since shrimp can be consumed undercooked or even raw (Dalsgaard et al., 1995). *Salmonella* spp. have also been isolated from commercial shrimp on numerous occasions (Gecan et al., 1994; Hatha and Lakshmanaperumalsamy, 1997; Heinitz et al., 2000; Iyer and Shrivastava, 1989; Llobrerra et al., 1990; Sumner, 1981).

Given the growing importance of aquaculture-produced shrimp, a quantitative microbial risk assessment (QMRA) for *Salmonella* spp. in shrimp is needed to determine the potential for microbiological health risks. In this study, a QMRA is conducted to 1) assess the microbiological health risks of using aquaculture for production of shrimp using *Salmonella* spp. as a reference pathogen; 2) assess the impact of industrial processing and consumer practices on shrimp *Salmonella* spp. risks for identifying risk drivers; 3) identify target *Salmonella* spp. concentrations for aquaculture ponds and health risk management options for aquaculture-produced shrimp; and 4) identify research gaps for improving the QMRA process related to shrimp consumption.

2. Methods

2.1. Hazard identification

Salmonella spp. was chosen as the index pathogen for this study; the use of representative reference pathogens of concern is an accepted practice in QMRA (Soller et al., 2017). *Salmonella* spp. are gram negative bacteria that are primarily transmitted by the fecal-oral route (Iwamoto et al., 2010; Mufty, 2008). At least 2500 different potentially pathogenic (to humans and animals) serotypes have been identified (Iwamoto et al., 2010; Mufty, 2008). The most common strain of *Salmonella* spp. isolated from shrimp is *Salmonella enterica* serovar Weltevreden (Koonse et al., 2005; Ponce et al., 2008; Uddin et al., 2015).

Aquaculture-produced seafood products are more likely contain *Salmonella* spp. than wild-caught seafood products (Koonse et al., 2005). The occurrence of *Salmonella* spp. in shrimp flesh was related to the presence of fecal bacteria in aquaculture ponds (Koonse et al., 2005; Sumner, 1981). The introduction of fecal bacteria may occur through wildlife waste (e.g., birds) or contaminated feed (Iwamoto et al., 2010). *Salmonella* spp. can also contaminate shrimp during processing and consumer food preparation (National Advisory Committee on Microbiological Criteria for Foods, 2008). In addition, processing contamination once seafood are removed from aquaculture ponds is thought to be the more common problem rather than contamination through aquaculture water itself (Edwards, 1992; Iwamoto et al., 2010). It is therefore important that the exposure models address *Salmonella* spp. contamination throughout the aquaculture and processing stages in order to determine their relative contributions to human health risks.

2.2. Exposure models

The process overview for shrimp growth and processing prior to arrival at the consumer is summarized in Fig. 1, beginning with the concentration of *Salmonella* spp. observed in a maturation pond. For purposes of comparing scenarios 1–8 and due to the lack of quantitative information on *Salmonella* spp. concentration in aquaculture ponds (Supplemental Table S1), the predicted concentration of *Salmonella* spp. in the aquaculture pond ($C_{Sa,pond,}$ [#/L]) was set to the WHO limit (10^3–10^4 *E. coli* per 100 mL) and converted to a *Salmonella* spp. concentration using a fecal indicator bacteria ratio (r_{pond}) as in Eq. (1).

$$C_{Sa,pond} = C_{EC,pond} r_{pond} \quad (1)$$

Consumer processing scenarios are summarized in Figs. 1 and 2. Processing scenarios 1–8 considered were computed according to Eq. (2) and are summarized in Table 1. The scenarios were:

1) Shrimp are gamma-irradiated, peeled and "deveined", and cooked properly ($a_{vein}R_{vein} = 0$), where "deveining" is defined as the removal of the shrimp digestive tract/gut;
2) Shrimp are gamma-irradiated, peeled and deveined, and undercooked ($a_{vein}R_{vein} = 0$);
3) Shrimp are gamma-irradiated, peeled-only, and cooked properly;
4) Shrimp are gamma-irradiated, peeled only, and undercooked;
5) Shrimp are not gamma-irradiated, peeled and deveined, and cooked properly ($a_{vein}R_{vein} = 0$);
6) Shrimp are not gamma-irradiated, peeled and deveined, and undercooked ($a_{vein}R_{vein} = 0$);
7) Shrimp are not gamma-irradiated, peeled-only, and cooked properly;
8) Shrimp are not gamma-irradiated, peeled only, and undercooked.

$$Dose_{consumer} = (a_{flesh}R_{flesh} + a_{vein}R_{vein})IC_{pond}e^{\sum_{i=1}^{n} k_i t_i} 10^{\sum_{i=1}^{n} -L_i} \quad (2)$$

Where log removals or growth are determined by a summation of first order coefficients (k_i) or $\log_{10}$ removal values (L_i) for process i where i = gamma irradiation, cooking, etc. and t is the time over which the first order process occurs. a_{flesh} *or* a_{vein} is the ratio of *Salmonella* spp. in [# / g] in each shrimp part compared to the pond water concentration [# per L] (final ratio units in L / g), R_p = the percentage of the total mass of shrimp contained in the flesh or vein (digestive tract) of the shrimp; and I is the daily intake rate of shrimp (g/per-day). An extensive literature review was conducted to parametrize this exposure model for scenarios 1 through 8. For scenarios where shrimp are peeled and deveined (1, 2, 5, and 6), $a_{vein}R_{vein} = 0$. Monte Carlo parameters for each scenario are summarized in Table 2.

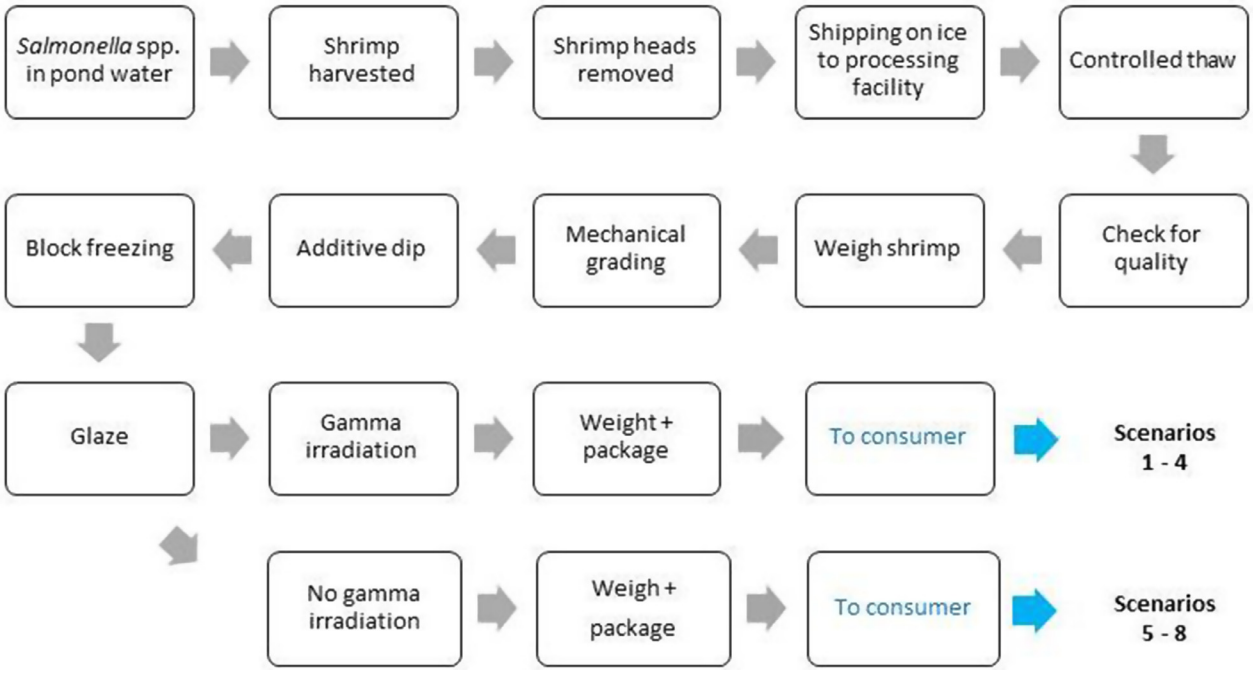

Fig. 1. Shrimp growth and processing prior to arrival at the consumer (continued in Fig. 2). Adopted from (Kanduri and Eckhardt (2008)) and FAO and WHO (2012).

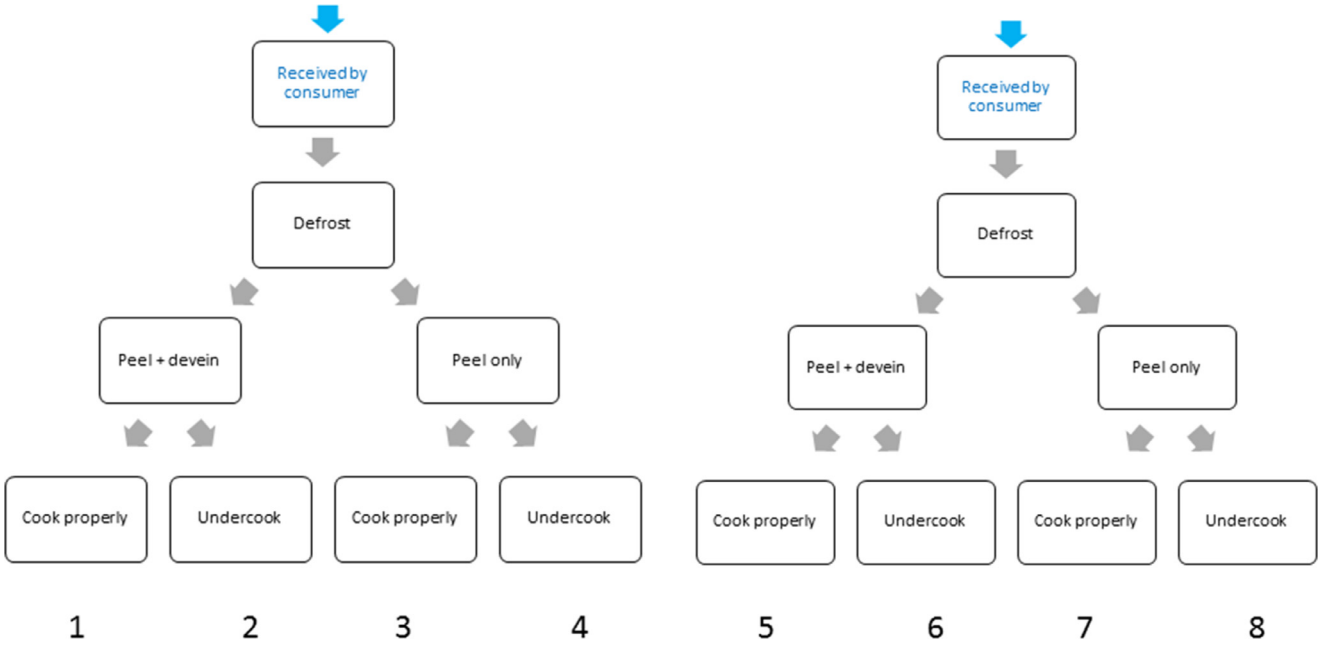

Fig. 2. Consumer processing for individuals consuming wastewater fed aquaculture-produced shrimp. These scenarios are considered for both gamma-irradiated and non-gamma-irradiated shrimp for a total of 8 scenarios labelled at the bottom of the figure and summarized in Table 1.

Table 1
Summary of processing scenarios evaluated.

Scenario	Gamma irradiation	Peel + devein	Peel only	Cook properly	Undercook
1	X	X		X	
2	X	X			X
3	X		X	X	
4	X		X		X
5		X		X	
6		X			X
7			X	X	
8			X		X

2.3. Dose response

The risk of an infection from different consumption scenarios ($P_{inf,daily}$) was calculated using the Beta-poisson dose-response formula for *Salmonella* spp. (Table 2) and using Eq. (3), where α and β are parameters of the Beta-Poisson dose response model (Haas et al., 1999). Dose response model parameters for pooled *Salmonella* nontyphoid strains from Haas et al. (1999) (p. 401) were used (α = 0.3126 and β = 2884). These parameters are derived from human feeding studies (McCullough and Eisele, 1951a; 1951b; McCullough and Elsele, 1951) using *Salmonella* nontyphoid strains (only dose response parameter point estimates were reported). The pooled analysis performed by the original authors included strains *S. enterica* serovar Newport, *S. enterica* serovar Derby, *S.enterica* serovar Bareilly, *S enterica* serovar Anatum strain I, *S enterica* serovar Anatum strain II, *S enterica* serovar Anatum strain III, *S. enterica* serovar Maleagridis strain I, *S. enterica* serovar Maleagridis strain II, and *S. enterica* serovar Maleagridis strain III. The endpoint from these experiments was infectivity, indicated by positive stool culture. The health endpoint for the dose response models is therefore *Salmonella* spp. infection.

$$P_{inf,daily} = 1 - \left(1 + \frac{Dose_{consumer}}{\beta}\right)^{-\alpha} \quad (3)$$

The probability of illness ($P_{ill,daily}$) is computed using a morbidity ratio in Eq. (4), $P_{ill}|P_{inf}$, defined using a distribution of morbidity ratios from Haas et al. (1999) with mean ± standard deviation of 41 ± 26% for multiple disease endpoints.

$$P_{ill,daily} = P_{inf,daily} P_{ill,daily}|P_{inf,daily} \quad (4)$$

2.4. Risk characterization

Annual infection risk was calculated as per Eq. (5), where n is the number of shrimp servings per year. $P_{ill,annual}$ was converted in the same way by substituting $P_{ill,daily}$ for $P_{inf,daily}$. The USEPA benchmark for annual infection risk associated with drinking water of $\leq 10^{-4}$ was used for comparison with computed annual infection risks, acknowledging that this is a conservative comparator that is used in the absence of other relevant risk benchmarks (Macler and Regli, 1993; Regli et al. 1991; USEPA, 1992).

$$P_{inf,annual} = 1 - \prod_{1}^{n} (1 - P_{inf,daily}) \quad (5)$$

The probability of illness was converted to a disability adjusted life year (DALY) metric using Eq. (6) (Lim et al., 2015). Lim and Jiang (2013) derived the DALY per case of illness of 6.14×10^{-3} for *Salmonella* spp. by considering the odds of severity, severity weight, and duration of illness for gastroenteritis, reactive arthritis, and inflammatory bowel disease resulting from infection. Lim and Jiang (2013) derived the values from a Netherlands population based on values available from previous work (Kemmeren et al., 2006; Vijgen et al., 2007), where the tolerable disease burden is 10^{-6} DALY per person per year (WHO, 2004). These values are thought to be comparable to a US population in the current analysis as other applicable values were not available.

$$DALY_{scenario} = \frac{DALY}{illness\ case} P_{ill,annual} \quad (6)$$

Additionally, a QMRA scenario was computed to solve for the concentration of *Salmonella* spp. associated with target infection (10^{-4}) and DALY (10^{-6}) risk targets. This was computed by simulating risks over a range of *Salmonella* spp. concentrations ($C_{Sa,pond}$). For each target risk, the median, 5th, and 95th percentiles for the target concentration were determined.

A sensitivity analysis was conducted to identify variables

Table 2
Monte Carlo input parameters.

Parameter	Symbol	Unit	Value	Distribution	Source
Concentration of *E. coli* spp. in aquaculture pond	$C_{EC,pond}$	# per L	Min = 10^4, Max = 10^5	Uniform	Simulated based on guidance values in (WHO, 2006)
Ratio of *Salmonella* spp. to *E. coli* in aquaculture pond	r_{pond}	# per L / # per L	10^{-4}	Point	(Brooks et al., 2005; Howard et al., 2007; Labite et al., 2010)
Ratio of *Salmonella* spp. in aquaculture pond to *Salmonella* spp. in shrimp digestive tract (vein)	$a_{digestivetract}$	# per g / # per L	shape = 0.255, scale = 3.941	Weibull	See supplemental materials and Section 2.2
Ratio of *Salmonella* spp. in aquaculture pond to *Salmonella* spp. in shrimp flesh (muscle)	a_{flesh}	# per g / # per L	Min = 4.62×10^{-5}, Max = 7.80×10^{-1}	Uniform	See supplemental materials and Section 2.2
Portion of total shrimp mass in flesh (muscle)	R_{flesh}	%	52.72	Point	(Al-Dagal and Bazaraa, 1999)
Portion of total shrimp mass in vein (digestive tract)	R_{vein}	%	0.4	Point	(Green, 1949)
Growth of *Salmonella* spp. during shipment on ice	k_{ice}	d^{-1}	Min = 0.18, Max = 2.23	Uniform	(Erdilal et al., 2014; Lalitha et al., 2010)
Duration of shipment	t_{ice}	d	2	Point	Assumption
Growth of *Salmonella* spp. during controlled thaw at processing plant	L_{ct}	Log_{10}	Min = 0, Max = 0.76	Uniform	(Wan Norhana et al., 2012)
Log removal of *Salmonella* spp. from additive dip	L_{dip}	Log_{10}	Min = 1.67, Max = 3.25	Uniform	(Shirazinejad et al., 2010)
Log reductions of *Salmonella* spp. from contact plate freezing and one week storage	L_{cf}	Log_{10}	$\mu = 1.26$, $\sigma = 0.10$	Normal	(Sommers et al., 2015)
Inactivation of *Salmonella* spp. from gamma irradiation (2.75- 3.5 KgY)	L_{irr}	Log_{10}	Min = 4.0, Max = 5.5	Uniform	(Abreu et al., 2009; Ito et al., 1993)
Growth of *Salmonella* spp. during consumer defrosting at room temperature	k_{cd}	d^{-1}	0.142	Point	(Erdilal et al., 2014)
Duration of consumer defrosting	t_{cd}	h	1	Point	(USDA, 2010)
Inactivation of *Salmonella* spp. from cooking	k_{cook}	s^{-1}	Min = −0.097, Max = –0.074	Uniform	(Brookmire et al., 2013)
Duration of proper cooking	$t_{cook,proper}$	s	$\mu = 96$, $\sigma = 8$	Normal	(Edwards et al., 2013)
Duration of undercooking	$t_{cook,under}$	s	15	Point	(Brookmire et al., 2013; USFDA, 2009)
Cooked shrimp intake per consumption event[b]	I	g/person-serving	85	Point	(USFDA, 2017)
Shrimp servings per year	n	Number per year	$\mu = 2.164$, $\sigma = 0.766$	Lognormal[a]	(USEPA, 2011)
Salmonella spp. infection dose response parameters	α	Unitless	0.3126	Point	(Haas et al., 1999)
	β		2884		
Morbidity ratio	$P_{ill}\|P_{inf}$	Fraction	$\mu = 0.41$, $\sigma = 0.26$; truncated(0,1)	Normal	(Haas et al., 1999)
Disability adjusted life years per case of illness	*DALY metric*	DALY/illness case	6.14×10^{-3}	Point	(Lim and Jiang, 2013)

[a]Lognormal parameters mean, standard deviation (μ, σ) calculated from population (normal) parameters ($\bar{x}$, s) using standard formulae as follows: $\mu = \ln(\bar{x}^2/(s^2+\bar{x}^2)^{1/2})$, $\sigma = [\ln(1 + (s^2/\bar{x}^2))]^{1/2}$, where $\bar{x}$ is the sample mean and s is the sample standard deviation. [b]Fish, shellfish, game meats, and meat or poultry substitutes: Entrees without sauce, e.g., plain or fried fish and shellfish, fish and shellfish cake.

contributing to uncertainty using 100,000 Monte Carlo iterations. All computations were performed in R (www.rproject.org) and using the mc2d package (Pouillot et al., 2015). The code for this model is available at https://github.com/DrKAHamilton.

The Spearman rank correlation coefficient was used to identify the most important predictive factors of annual infection risk and the DALY metric, where 0 is no influence and −1 or +1 indicates the output is wholly dependent on that input parameter. The model inputs were ranked based on their correlation coefficient with the output variable, annual infection risk or DALYs.

3. Results

3.1. Literature review results

3.1.1. Salmonella spp. in aquaculture ponds and accumulation in shrimp

To model *Salmonella* spp. accumulation in shrimp, studies that measured *Salmonella* spp. in aquaculture pond water and in different parts of shrimp after growth in an aquaculture system were reviewed, with the goal of modelling accumulation rates specific to different parts of the shrimp that were differentially treated during industrial and consumer processing steps. A significant relationship between fecal indicator bacteria (fecal coliforms and *E. coli*) concentrations in grow-out pond water and presence of *Salmonella* spp. in the shrimp product ($p = 0.003$) was previously observed (Koonse et al., 2005), supporting that bacteria in aquaculture grow-out water can become attached to- and/or internalized within- shrimp. Limited information is available where paired measurements of *Salmonella* spp. were made in both the shrimp and aquaculture pond water (Bhaskar et al., 1998; Faridullah et al., 2016; Koonse et al., 2005; Leangphibul et al., 1986; Lekshmy et al., 2014; Putro et al., 1990; Reilly and Twiddy, 1992; Wan Norhana et al., 2001), and quantitative information was very scarce, with ten studies quantifying various bacterial groups in water and shrimp and only one study described in two publications providing colony counts for *Salmonella* spp. in pond water and shrimp (Bhaskar et al., 1998; Bhaskar et al., 1995) (Supplemental Table S1). However, this study did not provide the volume of total sampled volume that was processed for microbiological parameters and therefore a ratio could not be computed for *Salmonella* spp. A ratio, *a*, was computed to relate the levels of various indicator bacteria reported in shrimp to levels of bacteria in the aquaculture pond water. The ratio was based on values reported in all studies where paired measurements (shrimp and pond water) were made and could be computed (Supplemental Table S1).

In most cases, due to traveling times between pond sites and processing locations and the tendency for leaving the shrimp heads on to degrade the final product, shrimp heads would be removed at the pond site. The most common type of shrimp sold in the United States is frozen raw shrimp with the shell on but head removed (Kanduri and Eckhardt, 2008), and consumers will typically peel but not necessarily de-vein the shrimp. Therefore, consumers would be exposed to either the shrimp flesh or the shrimp flesh and vein. Ratios were highest for shrimp digestive tract, followed by the other portions of the shrimp (muscle, muscle and shell, or whole shrimp), supporting that bacteria accumulates to a higher degree in the digestive tract. Only two studies made side by side comparisons of bacteria in water, shrimp digestive tract, and muscle (Phatarpekar et al., 2002; Shakibazadeh et al., 2009) and noted up to 4 orders of magnitude higher bacteria load in the gut compared to the shrimp flesh (muscle) for various bacteria.

Despite the relative concentration of bacteria in the shrimp gut, the gut comprises a small portion of the shrimp by mass (0.4% by mass) (Green, 1949) and therefore can represent a lesser portion of the total bacterial load (Al-Dagal and Bazaraa, 1999; Lalitha and Surendran, 2006b; Thampuran and Gopakumar, 1990) compared to estimates of mass of the head/gills (35–40% by mass) (Al-Dagal and Bazaraa, 1999; Green, 1949; Lalitha and Surendran, 2006b), shell (10.58% by mass), or flesh (52.72% by mass) (Al-Dagal and Bazaraa, 1999). As a result, accumulation ratio distrituions were fit to values from separate studies that reported processing the shrimp flesh (muscle) and those that reported processing the vein. This resulted in a best fit for $a_{digestive\ tract}$ of a Weibull distribution with shape = 0.255, scale = 3.941 (estimated mean = 84.2 # per g/ # per L). The best fit for a_{flesh} was a uniform distribution with minimum = 4.62×10^{-5} and maximum = 7.80×10^{-1} # per g/ # per L.

3.1.2. Shrimp processing

After harvest, shrimp are transported on ice to a processing facility (Kanduri and Eckhardt, 2008). No data were available for *Salmonella* spp. growth in shrimp stored on ice. Four studies (Erdilal et al., 2014; Lalitha and Surendran, 2006a; Lalitha et al., 2010; Okpala et al., 2014) measured microbial bacterial indicator growth for shrimp stored on ice. First order growth rates (k) computed for the current work using data from these studies ranged from 0.18 d^{-1} ($R^2 = 0.95$) to 2.23 d^{-1} (Supplemental Fig. S1). Average transport times will depend on the proximity of aquaculture facilities to processing facilities, with transport times minimized to prevent degradation of the product. For this analysis, a 2 d transport time was considered.

Following arrival at a processing facility, shrimp are typically stored for controlled thawing at 4 °C. Wan Norhana et al. (2012) measured the growth of *Salmonella enterica* serovar Typhimurium during storage at 4 °C and noted a 0.76 log increase in mean *Salmonella* spp. counts over 7 days of storage. Only three data points were available at day 0, 3, and 7 of storage. The increase in *Salmonella* spp. count did not occur until day 7. The exact controlled thaw time for a typical shrimp process was not available, therefore, a uniform distribution of 0–0.76 log10 increase in *Salmonella* spp. count was used in the model.

After thawing, shrimp are checked for quality, and weighed using a continuous weighing system prior to mechanical grading (FAO and WHO, 2012; Kanduri and Eckhardt, 2008). The shrimp are then dipped into an additive solution typically containing one or more compound including sodium metabisulfate, sodium benzoate, or sodium polyphosphates to prevent moisture loss, extend shelf-life, and preserve flavour prior to freezing (Kanduri and Eckhardt, 2008). In a study of Florida shrimp, after a 10-min dip in a 1.25% solution of sodium bisulfite, the recommended dip for shrimp, 67% (0.17 log) of aerobic bacteria were removed (Pyle and Koburger, 1984). However, this removal will vary with the type and concentration of preservative solution used and up to 2-log removal of psychotrophic bacteria for shrimp has been reported (Pardio et al., 2011). Furthermore, this is likely to differ depending on the individual pathogen. The only study available for *Salmonella* spp. demonstrated a mean of 1.67- log removal from shrimp during dip into a water control and 3.25 log removal with a 1.5% (v/v) lactic acid treatment (Shirazinejad et al., 2010) for whole homogenized shrimp. A uniform distribution for preservative dip removal ranging from 1.67 to 3.25 log removal was assumed. It was assumed the dip would inhibit growth on all portions (shell, flesh, etc.) of the shrimp.

Block freezing is the most common freezing method for raw, shell-on, headless shrimp and involves the shrimp being frozen between two plates (Kanduri and Eckhardt, 2008). The shrimp would then be glazed with a water spray to prevent clumping (FAO and WHO, 2012; Kanduri and Eckhardt, 2008). Gamma irradiation is not a common practice but is currently under consideration for improving the safety of crustaceans in the US and is therefore considered as an additional processing scenario; the maximum permitted dose is 6 kGy (USFDA, 2014). Previously a 3.5 kGy irradiation dose has been recommended (Ito et al., 1993) but the expected average dose in commercial irradiation is 2.75 kGy (Government of Canada, 2003). Doses between 2.75 kGy and 3.5 kGy will reduce *Salmonella* spp. in shrimp by 4–5.5 $\log_{10}$ (Abreu et al., 2009; Ito et al., 1993), therefore, a uniform distribution of these values was used. After irradiation, the shrimp are then weighed and packed for shipment to the consumer.

3.1.3. Consumer processing

Once arriving at the consumer, shrimp are defrosted prior to preparation. A conservative scenario is assumed where the consumer defrosts the shrimp at room temperature (25 ± 10 °C) rather than at the recommended temperature by USFDA (4 °C). To thaw 1 lb of frozen seafood will take approximately 1 h (USDA, 2010). Erdilal et al. (2014) measured total mesophilic count (TMC) grown at 37 °C in shrimp over a 12-day period in air at room temperature. Using data from this work, a growth constant (k) of 0.142 h^{-1} was calculated and assumed to be representative of *Salmonella* spp. (Supplemental Fig. S2).

While some recipes will call for shrimp with the tail shell left on, it is assumed that most consumers will peel the shrimp prior to consumption. "Deveining" the shrimp by running a knife along the dorsal side of the shrimp and removing the gut is a common consumer practice (Kanduri and Eckhardt, 2008). However, not all consumers will remove the digestive tract of the shrimp and this is considered as a scenario.

Finally, although in some cases shrimp can be eaten raw or acid-treated in ceviche, for example, only cooked and undercooked (improper) scenarios are considered. Boiling shrimp until they float to the surface of the water is a common method of cooking, accompanied by a colour change from grey to pink (Edwards et al., 2013). Edwards et al. (2013) reported the time for shrimp to float from boiling as 96 ± 8 s for an individual shrimp and 105 ± 2 s for 1 lb shrimp; the individual shrimp time was used. For undercooking, a 15 s cook time was used based on the recommendation to cook fish fillets to an internal temperature of 63 °C or higher for 15 s; although this is the time for the internal temperature of shrimp to remain at 63 °C before consumption, this appeared to be a reasonable estimate for undercooking due to the potential for misinterpretation that 15 s is appropriate for the total cook time (Brookmire et al., 2013; USFDA, 2009). The inactivation rate due to boiling was calculated assuming first order decay using data from inactivation of *Salmonella* spp. during boiling of extra-jumbo ($k = -0.097$) or colossal ($k = -0.074$) shrimp (Supplemental Fig. S3).

The USEPA Exposure Factors Handbook (2011) reports the number of servings of fish consumed per year from a study of New Jersey consumers over a 7-day recall period (Stern et al., 1996). The median and 95th percentile for the number of servings per week was 1.24 and 4.37, respectively (USEPA EFH Table 10–21). The same study (USEPA EFH Table 10–22) reported that 13.5% of the total reported meals (n = 1,447) were comprised of shrimp. To compute the number of servings per year of shrimp, the weekly number of servings was multiplied by 52 and 0.135 for a mean and 95th percentile of 8.705 and 30.677 servings per year, respectively (lognormal mean and standard deviation of 2.164 and 0.766). The recommended serving size for cooked shrimp is 85 g (USFDA, 2017).

3.2. Simulation results

3.2.1. Comparison of shrimp exposure scenarios

A comparison of *Salmonella* spp. doses at the point of exposure for each scenario are shown in Fig. 3a. Median doses ranged from 8.35×10^{-10} CFU (Scenario 1) to 7.02×10^{-2} CFU (Scenario 8). Annual infection risks from shrimp exposure scenarios 1–8 are shown in Fig. 3b. Median risks ranged from 8.03×10^{-13} (Scenario 1) to 6.58×10^{-5} (Scenario 8). 95th percentile risks ranged from 3.66×10^{-11} (Scenario 1) to 1.85×10^{-3} (Scenario 8). The 95th percentile for scenario 6 and 8 exceeded an annual infection risk target of 10^{-4}. The greatest difference between annual infection risks for the scenarios was due to proper vs. improper cooking (5–6 orders of magnitude) and gamma irradiation (4–5 orders of magnitude) compared to (less than 1 order of magnitude) peeling and deveining vs. peeling only.

The DALY metric for scenarios 1–8 are shown in Fig. 3c. The median DALY metric ranged from 1.75×10^{-15} to 1.49×10^{-7} DALYs per person per year. While the median values did not exceed the target DALY metric (10^{-6}), the 95th percentile values ranged from 9.50×10^{-14} to 4.89×10^{-6} and would exceed the target DALY metric for scenarios 6 and 8.

3.2.2. Concentration targets for salmonella spp. in aquaculture pond water

Given that the existing WHO guideline value of 10^3–10^4 *E. coli* per 100 mL would be insufficient in some cases to protect for *Salmonella* spp. health risks, concentrations necessary to satisfy the target risk conditions (10^{-4} annual probability of infection or 10^{-6} DALYs per person per year) were determined (Table 3). Target concentrations in aquaculture pond water would vary substantially depending on the industrial and consumer processing scenarios, with median *Salmonella* spp. concentration targets ranging from 6.02×10^8 (95% CI 1.56×10^7, 4.43×10^{10}) to 5.45×10^0 (95% CI 2.41×10^{-1}, 1.85×10^2) per L for the annual infection risk target and 3.19×10^9 (95% CI 5.50×10^7, 2.02×10^{11}) to 3.05×10^1 (95% CI 1.00×10^0, 1.45×10^3) per L for the DALY target.

3.2.3. Sensitivity analysis

The results of the sensitivity analysis are shown in Fig. 4. For all scenarios, the log removal due to the additive dip (L_{dip}) was the most influential predictor variable (Spearman rank correlation coefficients ranging from −0.42 to −0.53 for annual infection risk as the outcome variable, and −0.41 to −0.50 for the DALY metric as the outcome variable). The second most influential variable depended on the scenario and was either the removal due to gamma irradiation (L_{irr}) (−0.40 to −0.45 for annual infection risk and −0.39 to −0.43 for the DALY metric), the growth constant during shipping on ice (k_{ice}) (0.33 to 0.41 for annual infection risk and 0.32 to 0.39 for the DALY metric), or the fraction of *Salmonella* spp. in the pond that accumulated in the shrimp flesh (a_{flesh}) (0.26 to 0.41 for annual infection risk and 0.25 to 0.39 for the DALY metric).

4. Discussion

There is currently no agreement regarding acceptable levels of *Salmonella* spp. in seafood, however, several countries including the United States and Australia have a zero tolerance policy for the presence of *Salmonella* spp. in both raw and ready to eat/cooked shrimp (Norhana et al., 2010). A QMRA was performed to assess the relative risks due to exposure to *Salmonella* spp. from consumption of shrimp raised in aquaculture ponds under a variety for industrial and consumer processing scenarios. The results of the scenario analysis indicate that improper cooking times in non-gamma-irradiated shrimp represent the highest annual infection risks and DALY per person per year. These findings support that consumer handling practices have a large impact on risks and that consumers should follow appropriate guidelines for cooking shrimp (Edwards et al., 2013). The concentration of *Salmonella* spp. in aquaculture ponds had only a moderate impact on each risk scenario, demonstrating that there may be other more effective management points for reducing risks. However, because management of pond water quality is of high concern for seafood producers, target concentrations were computed to inform the choice of treatment options. The target concentrations varied widely depending on the processing chain, highlighting the importance of producers understanding downstream processes in order to choose appropriate risk management interventions. As it is difficult to ensure correct consumer handling processes, and especially in the absence of gamma irradiation, it is prudent to restrict aquaculture pond limits for *Salmonella* spp. to low levels (median 5–30 *Salmonella* spp. per L in pond water for scenarios 6 and 8, where undercooking and lack of consumer "deveining" occurred).

Limited risk assessments have been performed for seafood consumption in order to inform risk management practices (Chanpiwat et al., 2016; Iwahori et al., 2010; Rico and Van den Brink, 2014; Sani et al., 2013; Yajima and Kurokura, 2008) and only two (Sani et al., 2013; Yajima and Kurokura, 2008) have focused on microbial risks, with one (Sani et al., 2013) examining *Vibrio parahaemolyticus* in tiger

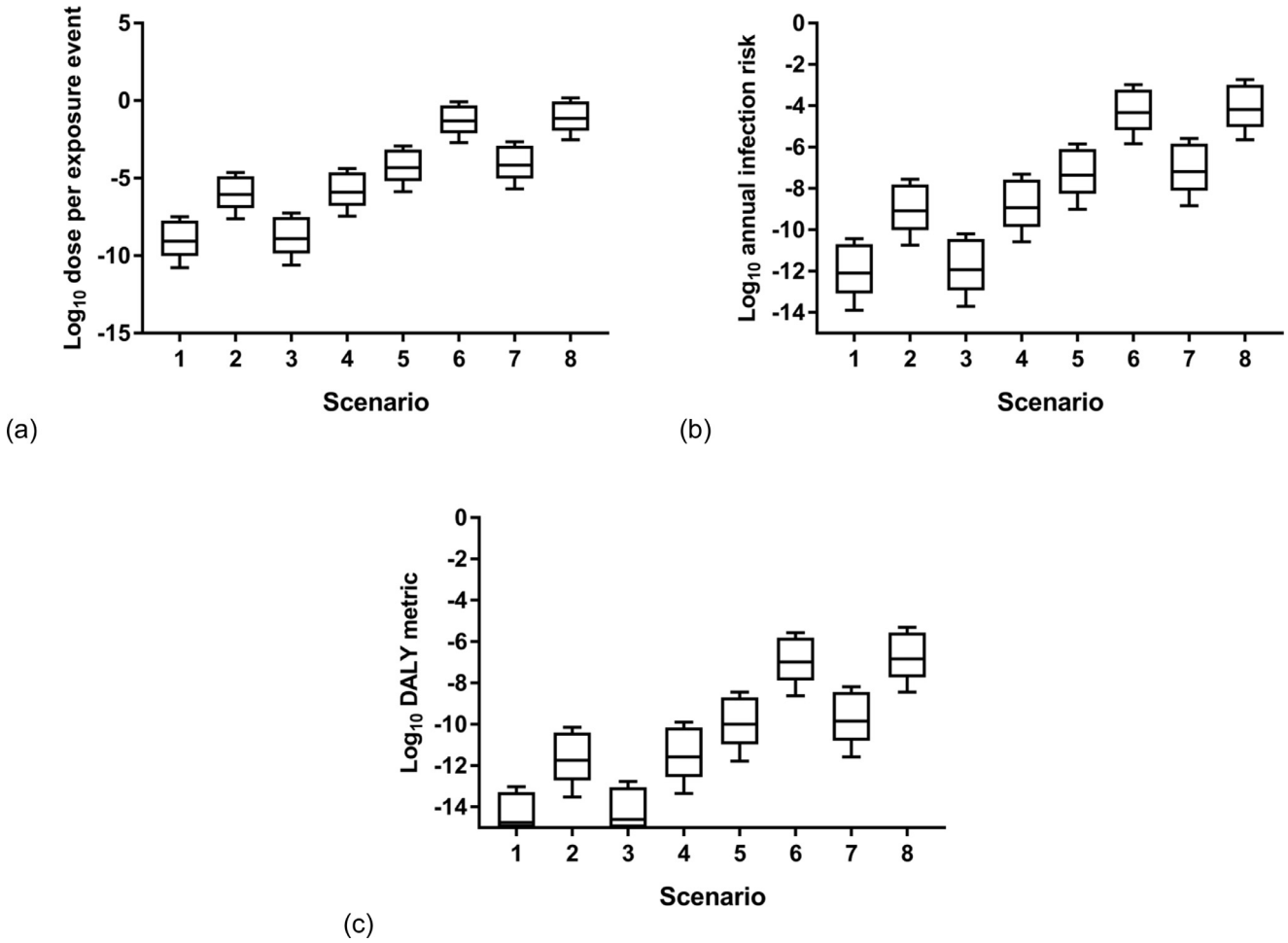

Fig. 3. (a) *Salmonella* spp. dose per exposure event; (b) Annual *Salmonella* spp. infection risk; (c) Disability adjusted life year (DALY) metric for all *Salmonella* spp. health endpoints. Bars shown represent the 5th, 25th, 50th, 75th, and 95th percentiles of each distribution.

Table 3
QMRA results for target concentrations necessary to incur a 10^{-4} annual infection risk or 10^{-6} DALY threshold for each shrimp processing scenario 1–8.

Scenario	Water quality target for *Salmonella* spp. infection 10^{-4} threshold Median (5th, 95th) [#/L]	Water quality target for *Salmonella* spp. DALY 10^{-6} threshold Median (5th, 95th) [#/L]
1	6.02×10^{8} (1.56×10^{7}, 4.43×10^{10})	3.19×10^{9} (5.50×10^{7}, 2.02×10^{11})
2	5.21×10^{5} (2.21×10^{4}, 2.20×10^{7})	2.67×10^{6} (1.02×10^{5}, 1.71×10^{8})
3	5.28×10^{8} (1.01×10^{7}, 2.21×10^{10})	1.79×10^{9} (3.37×10^{7}, 1.49×10^{11})
4	3.50×10^{5} (1.18×10^{4}, 1.66×10^{7})	1.80×10^{6} (4.01×10^{4}, 1.54×10^{8})
5	1.17×10^{4} (3.45×10^{2}, 3.57×10^{5})	5.15×10^{4} (1.50×10^{3}, 2.56×10^{6})
6	1.09×10^{1} (4.94×10^{-1}, 2.71×10^{2})	6.01×10^{1} (1.74×10^{0}, 1.94×10^{3})
7	1.04×10^{4} (2.17×10^{2}, 2.72×10^{5})	3.37×10^{4} (1.11×10^{3}, 1.96×10^{6})
8	5.45×10^{0} (2.41×10^{-1}, 1.85×10^{2})	3.05×10^{1} (1.00×10^{0}, 1.45×10^{3})

shrimp. The incidence rate calculated by Sani et al. (2013) was 1.3 illnesses/100,000 population/year (aged between 18 and 59 years) while the 90% distribution was between 0.5 and 2 based on a probability of illness of 4.8×10^{-6} for a single cooked shrimp meal. These annual risks (median probability 1.3×10^{-5}) are comparable with those calculated in the current study for scenarios 6 and 8. Estimates for farming and handling finfish by Yajima and Kurokura (2008) in excreta-based systems were 17.84 and 1.63 per 10,000 people exposed, compared to 11.01 and 0.16 per 10,000 people exposed in feed-based systems, respectively. This is not calculated on an annual basis and therefore it is challenging to compare with the estimates generated here given different exposure patterns and an occupational population.

The primary goal of the current QMRA was to compare differences in industrial shrimp processing methods for identifying microbial risk drivers. However, it is noted here that the current study did not consider health risks from chemicals, metals, or other health stressors that could be present in aquaculture-produced seafood which may in some cases present a greater long-term risk. The focus on microorganisms was chosen as these acute risks are considered the driving human health risk concern (Strauss 1996), however in the future a more comprehensive assessment could consider a full suite of health risks in this context.

In the models explored for this work, several other limitations could be addressed and key processes could be incorporated to provide a more detailed consideration of shrimp processing in future assessments. The current assessment did not consider the fate and transport of bacteria in aquaculture ponds and sediments; few studies reported the time of shrimp harvest, and more information is needed to assess the

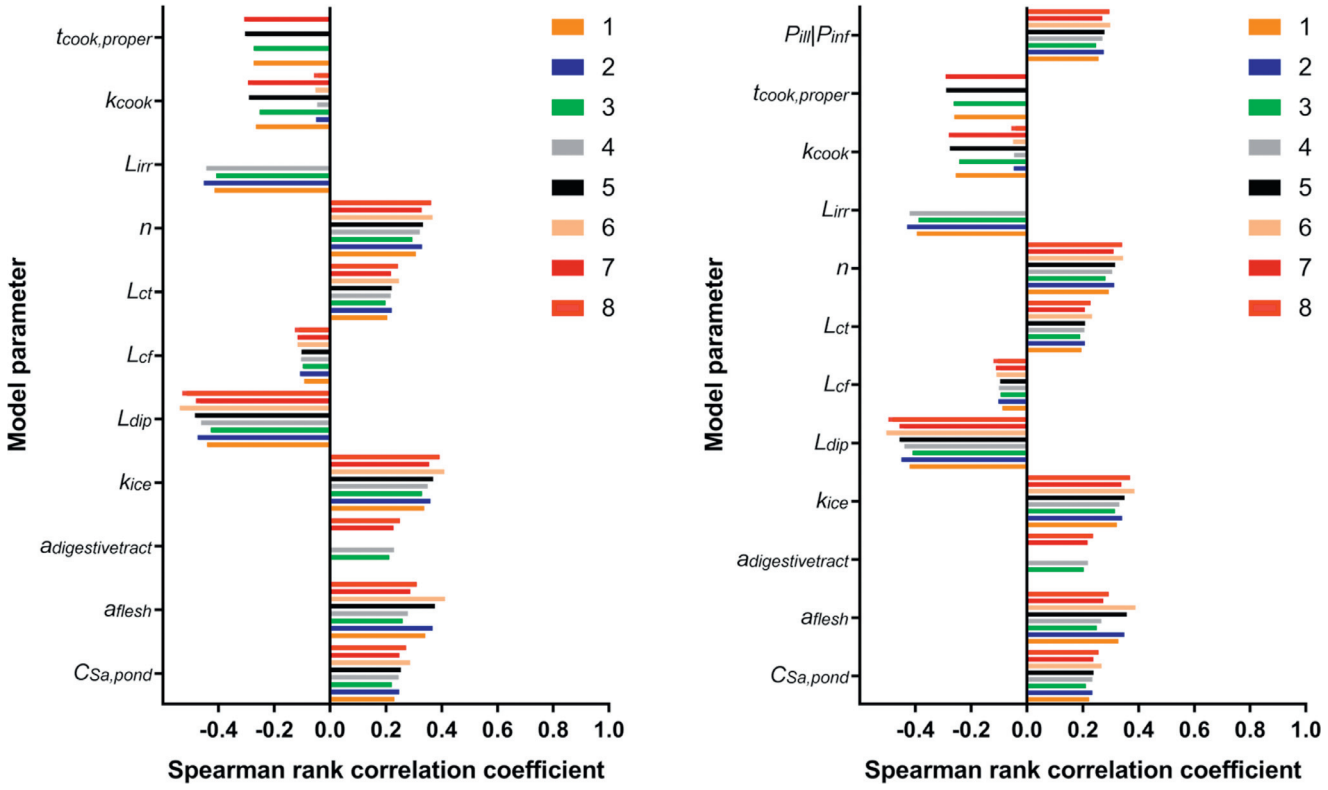

Fig. 4. Sensitivity analysis with Spearman rank correlation coefficients for annual *Salmonella* spp. infection risk (left) and DALY metric (right).

relationship between concentrations of bacteria in aquaculture ponds, water quality parameters (pH, salt content, dissolved oxygen, etc.), and the time shrimp are kept in the pond to achieve maturation. The time spent by shrimp in the pond and therefore their development are related to size, exoskeleton molting, and shedding of the gut lining, which may also play a role in bacterial densities in various shrimp organs (Dempsey et al., 1989). Additionally, shrimp feed, wildlife, and cross-contamination from harvesting/processing workers were not considered to provide additional sources of *Salmonella* spp. in the system.

Shrimp metabolic rates and transfer between organs can be affected by various temperature and water quality conditions (Alday-Sanz et al., 2002), and could change based on variations in these variables. Migration of bacteria between the organs of shrimp during processing was also not considered. The pathogen accumulation rates calculated in this study are likely to be specific to the species of shrimp considered (i.e. Paneid shrimp), and could vary for other types of shrimp species. Additionally, the studies modelled for pathogen accumulation were based primarily on fecal indicator bacteria and were assumed to bioaccumulate similarly in shrimp to *Salmonella* spp. due to lack of organ-specific accumulation information for *Salmonella* spp.. In the absence of more detailed information, it was assumed for this assessment that decay or growth rates for *Salmonella* spp. were identical in all parts of the shrimp; additional information in this area could help to identify other driving factors in the risk assessment.

Previous work has indicated that contamination after seafood are removed from aquaculture ponds can be a significant driver of pathogen risks (Edwards, 1992; Iwamoto et al., 2010). For example, a *Salmonella* spp. outbreak in shrimp was previously linked to facilities where hand-processing of shrimp is used in India (Elsea et al., 1971). Cross-contamination during industrial shrimp processing and consumer handling and contact with kitchen surfaces was not considered; however, contamination of processing equipment surfaces and transfer to shrimp can play an important role in pathogen risks (Guobjoernsdottir et al., 2005). Temperature control may vary throughout shrimp handling, further adding to variability during shrimp processing and transport to the customer (Sumner et al., 1982).

Transfer from shrimp to consumer kitchen surfaces could be incorporated into more detailed considerations of consumer behaviour using bacterial transfer rates as have been previously applied for chicken (Carrasco et al., 2012). Biofilms on the surfaces of seafood can impact both die-off /growth as well as transfer to surfaces and was not considered (Mizan et al., 2015). Additionally, other shrimp cooking methods such as baking could be considered (Brookmire et al., 2013).

Salmonella spp. contamination in shrimp and other seafood products poses both a public health risk as well as an economic burden associated with lost productivity due to illnesses and increased resource requirements for monitoring. The FDA is required to sample and analyse a subset of products for contamination, as well investigate sources and causes for outbreaks in order to minimize consumer exposure (Koonse et al., 2005). Shrimp are an important aquaculture product and potential management options can assist in mitigating the risk of *Salmonella* spp. exposure from wastewater-fed aquaculture practices. Primarily, emerging treatment options, such as gamma irradiation, show promise for ensuring the suitability of wastewater-fed shrimp aquaculture. Other practices include: monitoring the concentration of *Salmonella* spp. in aquaculture ponds such that it does not exceed an established threshold; protecting the aquaculture ponds from external sources such as birds and other animals; permitting sunlight to reach the ponds to assist in photoinactivation of potentially harmful pathogens; and educating and training workers on how to properly handle shrimp in order to minimize cross-contamination of the product when harvesting and transferring to freezing facilities. These recommendations can be integrated into the existing Hazard Analysis Critical Control Point (HACCP) plans recommended for shrimp cultivation. The HACCP plans are designed to promote guidelines that will prevent, eliminate or reduce food safety hazards to within an acceptable level. Increasing attention to these aspects can mitigate health risks while promoting the use of aquaculture to meet food security needs.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2018.04.001.

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