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The seaweed fly (Coelopidae) can facilitate environmental survival and transmission of *E. coli* O157 at sandy beaches

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ABSTRACT

The sustainable management of recreational beaches is essential for minimising risk of human exposure to microbial pathogens whilst simultaneously maintaining valuable ecosystem services. Decaying seaweed on public beaches is gaining recognition as a substrate for microbial contamination, and is a potentially significant reservoir for human pathogens in close proximity to beach users. Closely associated with beds of decaying seaweed are dense populations of the seaweed fly (Coelopidae), which could influence the spatio-temporal fate of seaweed-associated human pathogens within beach environments. Replicated mesocosms containing seaweed inoculated with a bioluminescent strain of the zoonotic pathogen *E. coli* O157:H7, were used to determine the effects of two seaweed flies, *Coelopa frigida* and *C. pilipes*, on *E. coli* O157:H7 survival dynamics. Multiple generations of seaweed flies and their larvae significantly enhanced persistence of *E. coli* O157:H7 in simulated wrack habitats, demonstrating that both female and male *C. frigida* flies are capable of transferring *E. coli* O157:H7 between individual wrack beds and into the sand. Adult fly faeces can contain significant concentrations of *E. coli* O157:H7, which suggests they are capable of acting as biological vectors and bridge hosts between wrack habitats and other seaweed fly populations, and facilitate the persistence and dispersal of *E. coli* O157:H7 in sandy beach environments. This study provides the first evidence that seaweed fly populations inhabiting natural wrack beds contaminated with the human pathogen *E. coli* O157:H7 have the capacity to amplify the hazard source, and therefore potential transmission risk, to beach users exposed to seaweed and sand in the intertidal zone. The risk to public health from seaweed flies and decaying wrack beds is

usually limited by human avoidance behaviour; however, seaweed fly migration and nuisance inland plagues in urban areas could increase human exposure routes beyond the beach environment.

Key words: biological vector; insect; public health; wrack; zoonotic pathogen

HIGHLIGHTS

Seaweed flies (Coelopidae) enhance survival of *E. coli* O157:H7 in seaweed and sand.

C. frigida flies vector *E. coli* O157:H7 to seaweed and sand.

C. frigida female flies ingest and excrete *E. coli* O157:H7 more rapidly than males.

Coelopidae are bridge hosts of *E. coli* O157:H7 in sandy beach environments.

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1. INTRODUCTION

Shiga-toxin (*stx*) producing *Escherichia coli* (STEC) serotype O157:H7 is often carried in the digestive tracts of various animal reservoirs including cattle and other ruminants [Ferens and Hovde, 2011]. Human infection by *E. coli* O157:H7 can cause acute gastrointestinal illness, presenting primarily in the form of diarrhoea, but can also cause haemolytic uremic syndrome (HUS) and lead to permanent liver damage [Griffin and Karmali, 2017]. Importantly, infection can be caused by extremely low infectious dose rates (< 10 - 50 viable cells), and can be fatal for young children or those with compromised immune systems [Teunis et. al., 2004, Lim et. al., 2010]. There is also growing concern about the multiple antimicrobial resistance of shiga toxin-producing *Escherichia coli*, due in part to indiscriminate application of antibiotics to livestock and the various direct and indirect pathways by which humans can become infected (Hoelzer et. al., 2017). Cattle, human, environmental and food sources of 129 *E. coli* O157:H7 isolates have exhibited resistance to at least five antimicrobials (Srinivasan et. al., 2007). Coupled with the increased risk of antibiotic dosing provoking HUS in clinical patients (Freedman et. al., 2016), there is an important public health risk posed by under-reported reservoirs and undocumented vectors of *E. coli* O157:H7 in the environment. Human *E. coli* O157:H7 infection most commonly occurs through consumption of contaminated food and water, person-to-person contact, or exposure to animal carriers [Kintz et. al., 2017]. The epidemiology of *E. coli* O157:H7 is shaped by multiple routes of exposure throughout the wider environment in which human-animal ecological niches overlap, which coupled with the specific survival characteristics of *E. coli* O157:H7 in non-host habitats prevents accurate prediction of the spatio-temporal fate of this pathogen in the environment [Chapman et. al., 2017, van Elsas et. al., 2011]. Hence, our incomplete understanding of the survival capacity of *E. coli* O157:H7 in hostile secondary environments, together with a lack of accurate quantification tools, hampers efforts to manage its public health risk [Quilliam et al., 2011, Young, 2016].

The level of risk of human infection by a zoonotic pathogen such as *E. coli* O157:H7 is partly determined by the prevalence of infection amongst disease reservoirs and secondary (bridge) hosts

[Lloyd- Smith et. al., 2009]. Important bridge hosts known to spread and transmit *E. coli* O157:H7 directly and indirectly to humans are synanthropic (e.g. houseflies) and non-synanthropic (e.g. fruit flies) species of fly (Diptera) [Pace et. al., 2017, Janisiewicz et. al., 1999]. Fly larvae are typically nutritionally dependent on bacteria in their diet, although destructive gut enzymes and antimicrobial substances enable the larvae of some species to produce near-sterile faecal excretions [Mumcuoglu et. al., 2001, Nayduch and Burrus, 2017]. The environment is the principal source of bacterial contamination of adult flies, and often occurs via direct ingestion from a feeding surface or indirectly during grooming [Nayduch and Burrus, 2017]. Thereafter, bacteria attached to the fly exoskeleton may be passively transferred to other surfaces, including from hairs, legs and adhesive feet, or deposited via regurgitation or faecal excretions if the bacteria are capable of surviving passage through the digestive tract [Sasaki et. al., 2000, Graczyk et. al., 2001, Sukontason et. al., 2006]. *E. coli* O157:H7 has been found to replicate on housefly mouthparts thus extending the duration of its expression in fly faeces, and to grow on house fly exoskeletons and in vomit spots [Kobayashi et. al., 1999, Wasala et. al., 2013]. The cumulative effect of these mechanical and biological interactions of flies with pathogens is to enhance their capacity for disease transmission.

Recreational beach environments are vulnerable to downstream transport of human pathogens, and virulence stx_2 genes of pathogenic *E. coli* have been isolated from swash zone sand of freshwater beaches (Cho et. al., 2016, Bauer and Alm, 2012). The source of an outbreak of *E. coli* O157:H7 infection amongst seven children playing on a UK marine beach, for example, was identified as a contaminated stream draining an area of upstream cattle grazing, recently subjected to heavy rainfall (Ihekweazu et. al., 2006). Although seawater and sand are known reservoirs of faecal bacteria [Solo-Gabriele et al., 2016], additional reservoirs for microbial pathogens within beach environments include decaying piles of seaweed (wrack), which can also enhance the persistence of *E. coli* in adjacent seawater and sand [Imamura et. al., 2011, Quilliam et. al., 2014]. Stranded, decaying wrack is thus a potentially important reservoir for *E. coli* O157:H7 and can concentrate human exposure risks within recreational spaces such as bathing water beaches. In

beach environments, the public often share their recreational space with seaweed flies (Coelopidae), which are attracted to decaying wrack beds within a few hours of deposition along the strandline [Dobson, 1974a]. Seaweed flies undergo their entire life-cycle within wrack beds, and often form dense populations. In northern Europe, the dominant species are *C. frigida* (Fabricius) and *C. pilipes,* and detached seaweed induces both male mating behaviour and female ovipositioning, with *C. frigida* preferentially laying eggs on *Laminaria* spp. and *C. pilipes* favouring *Fucus* spp. [Dobson, 1974a, Edward et. al., 2007, Dunn et. al., 2002]. Although the potential for decaying wrack beds to function as reservoirs of human pathogenic bacteria is gaining recognition [Quilliam et. al., 2014, Russell et. al., 2014], there are no published studies addressing the risk of seaweed flies disseminating human pathogens between wrack habitats.

Identification of all possible modes of direct and indirect transmission of human microbial pathogens in the coastal zone will enable more effective management of the potential public health risk in that environment [Young, 2016, Caron et. al., 2015]. Therefore, the aim of this study was to establish whether *C. frigida* and *C. pilipes* can influence the survival and transmission dynamics of *E. coli* O157:H7. Furthermore, the use of a chromosomally *lux*-marked (Tn5 *lux*CDABE) E. coli O157:H7 serotype (Ritchie et. al., 2003) provided the opportunity to measure bioluminescence of the pathogen as a proxy for changes in its metabolic activity in decaying seaweed and in sand in the presence of flies and larvae, and in response to ingestion by both life stages. Specifically, the objectives were to determine whether the presence and feeding activity of multiple generations of flies and larvae respectively and of both species had consequences for the persistence and metabolic activity of *E. coli* O157:H7 on decaying seaweed and in beach sand; determine the effect of *C. frigida* larval feeding, developmental stage and larval-associated native microbiota, and the competitive effect of natural wrack bed bacterial communities, on the survival and metabolic activity of *E. coli* O157:H7 in the larval gut, on decaying seaweed and in beach sand; establish the capacity for *C. frigida* flies to transmit, and function as bridge hosts of, *E. coli* O157:H7, investigate whether vector competence differed between females and males, and determine the metabolic activity of the

vectored pathogen, and finally to quantify the contribution of faecal excretion of metabolically active *E. coli* O157:H7 to transmission by *C. frigida* adults following pathogen ingestion, and identify whether capacity for biological transmission differed between females of different reproductive stage and males. It was hypothesised that (i) the presence of seaweed flies and larvae facilitates the persistence and activity of *E. coli* O157:H7 in wrack beds and underlying sand; (ii) larval feeding suppresses *E. coli* O157:H7 populations and activity in their seaweed substrate by inactivating the pathogen during larval digestion, that this mode of action is mediated both by larval developmental stage and the presence of native gut and exoskeleton bacteria, and that natural bacterial assemblages in wrack beds limit *E. coli* O157:H7 growth through competition; (iii) *C. frigida* flies, particularly females, are a bridge host and transmission pathway for metabolically active *E. coli* O157:H7, and (iv) metabolically active *E. coli* O157:H7 can be dispersed and survive in the environment via biological transmission in faecal excretions, females exhibit a greater capacity for this mode of transmission than do males, and females with developing eggs imbibe more liquid than females with mature eggs.

2. METHODS

2.1 Preparation of Coelopidae colonies.

Colonies of *C. frigida* and *C. pilipes* were cultured from wild larvae collected from stranded wrack beds on an exposed and natural sandy beach in Fife, Scotland (56°11.191'N, 2°48.679'W). Larvae were grown in a controlled environment cabinet (Reftech B.V., Netherlands) at 25 °C ± 2 °C, a relative humidity of 60 % and a photoperiod of 12 h, and fed with fresh, finely minced (0.5 cm^2) seaweed species characteristic of a stranded wrack bed: (*Laminaria digitata* (Hudson) (40 %), *Laminaria hyperborea* (Gunnerus) (20 %), *Fucus serratus* (L.) (20 %), *Ascophyllum nodosum* (L.) (10 %), *Saccharina latissima* (L.) (5 %), *Palmaria palmata* (L.) (3 %) and *Rhodomela confervoides* (Hudson) (2 %). Newly emerged adults were collected as virgins twice daily through attraction to a light box. Following 10 s anaesthesia with CO_2, flies were classified by species and sex, and stored at 4 °C in

ventilated 150 ml plastic Erlenmeyer flasks containing cotton wool soaked in a 50 % glucose solution; all flies were used in experimental mesocosms within 96 h.

2.2 . **Experimental design**.

A total of four experiments were conducted. Three utilised mesocosms containing multiple individuals designed to investigate Coelopidae population level interactions with *E. coli* O157:H7 in simulated wrack bed habitat comprising decaying seaweed and underlying sand. In the first study, (i) *C. frigida* and *C. pilipes* flies were introduced to mesocosms to determine the effect of mixed species colonies (and multiple generations of flies and larvae) on *E. coli* O157:H7 persistence and activity in wrack bed habitat over several months. The second mesocosm experiment (ii) sought to examine the effect of *C. frigida* larval feeding and development on *E. coli* O157:H7 persistence in simulated wrack bed habitat, the facilitatory role of the larvae's native exoskeleton and gut microflora on their capacity to digest the pathogen, and the competitive effect of natural wrack bed bacterial communities on *E. coli* O157:H7. The third mesocosm study (iii) was designed to investigate whether *C. frigida* flies were capable of transmitting *E. coli* O157:H7 between wrack bed habitats. A fourth experiment (iv) employing microcosms containing single adult individuals fed known concentrations of *E. coli* O157:H7 was intended to quantify at fine scale the role of biological transmission of the pathogen by the flies to their vectoring capability.

2.3 Materials for experimental mesocosms.

Seaweed, sand and seawater were collected at low tide the day before starting each experiment. Recently deposited seaweed (*Laminaria* spp. (70 %) and *Fucus* spp. (30 %)), was gathered from the strandline; sand was collected from above the drift line and seawater from the surf zone. All environmental materials were stored at 4 °C prior to transfer to mesocosms. Background *E. coli* and total heterotrophic bacteria (THB) were enumerated in all seaweed, sand and seawater used in experimental mesocosms, and pH and water content measured in the seaweed and sand (Table 1).

To quantify background *E. coli* and THB concentrations, four replicate samples of 10 g of seaweed or 5 g of sand were added to 10 ml or 5 ml of sterile seawater, respectively, and then vortexed for 1 minute. The supernatant was subsequently serially diluted with sterile seawater and 50 µl streaked onto Membrane Lactose Glucuronide Agar (MLGA) (CM1031, Oxoid) to enumerate presumptive *E. coli*, or R2A agar (CM0906, Oxoid) to enumerate THB. Seawater samples (n = 4) were shaken and 100 ml vacuum-filtrated through a 0.45 µm cellulose acetate membrane (Microsart CN-filter, Sartorius Stedim Biotech GmbH, Goettingen, Germany) and transferred onto MLGA. Plates for *E. coli* were incubated at 37 °C for 24 h and plates for THB incubated at 18 °C for 48 h. For sand and seaweed samples, bacterial concentrations were expressed as CFU (colony forming units) g^{-1} dry matter content (where representative seaweed and sand samples were dried at 80 °C for 24 h), or expressed as CFU 100 ml^{-1} for sea water samples.

Experiment	Environmental parameter	Seaweed	Sand
1*	Water content (%)	74 ± 1	14 ± 0.2
	pH	-	9.6 ± 0.1
	E. coli (CFU g^{-1})	0	0
	Total heterotrophic bacteria (CFU g^{-1})	33 x 10^3 (± 0.23)	52 ± 0.44
2 and 3+	Water content (%)	81 ± 0.2	12 ± 0.4
	pH	-	8.0 ± 0.2
	E. coli (CFU g^{-1})	< 10	0
	Total heterotrophic bacteria (CFU g^{-1})	110 x 10^4 (± 0.36)	20 ± 0.13

* *E. coli* O157:H7 persistence

+ *E. coli* O157:H7 survival during larval development and *E. coli* O157:H7 transmission by flies

Table 1. Characteristics of seaweed and sand used in mesocosm experiments. Values represent the means ± SE.

A non-toxigenic, chromosomally *lux*-marked (Tn5 *lux*CDABE) *E. coli* O157:H7 serotype [Ritchie et. al. 2003] was grown on Sorbitol MacConkey Agar (SMAC) (CM0813, Oxoid) supplemented

with cefixime and potassium tellurite (CT) (SR0172, Oxoid) at 37 °C for 24 h for the selective isolation of *E. coli* O157:H7. Presumptive colonies of *E. coli* O157: H7 were confirmed by a latex agglutination test (DR0260, Oxoid), and then grown in Luria-Bertani (LB) broth (CM1018, Oxoid) at 37 °C, at 100 rev min^{-1}, for 18 h. Cells were washed three times in phosphate buffered saline (PBS) and re-suspended in PBS in preparation for use in experimental mesocosms. The bioluminescence phenotype of the *lux* biomarker of this strain of *E. coli* O157:H7 is dependent on the cellular energy status. As cellular metabolism requires energy, bioluminescence output can be used as a proxy for the metabolic activity of the population of cells, and thus, bioluminescence allows a quick *in situ* estimation of how metabolically active the *E. coli* O157:H7 population is [Quilliam et al., 2012].

2.4 Persistence and activity of *E. coli* O157:H7 in the presence of Coelopidae colonies.

This mesocosm experiment was designed to quantify the effect of the presence of multiple generations of *C. frigida* and *C.pilipes* flies and larvae on *E. coli* O157:H7 persistence and metabolic activity in seaweed and sand. A total of 8 mesocosms were established, each consisting of a 5 L plastic container (Addis Ltd., UK) with paper towelling secured over a hole (10 cm x 10 cm) in the lid to allow gas exchange. The mesocosms contained the following treatments: (A) both *E. coli* O157:H7 and flies present; (B) just *E. coli* O157:H7 with no flies.. Each treatment consisted of four replicate mesocosms, and each mesocosm contained 1 kg of finely minced (0.5 cm^2) seaweed (approximately 5- 6 cm depth) laid over 2 kg of sand (approximately 3 cm depth). For mesocosms containing *E. coli* O157:H7, the inoculant was mixed with seaweed for 5 min in a stomacher bag with 200 ml of seawater contaminated with *E. coli* O157:H7 (1.84 x 10^9 CFU ml^{-1}). Seaweed added to the control mesocosms (those not containing *E. coli* O157:H7) was mixed with 200 ml of uncontaminated, unsterilised seawater. To each mesocosm containing flies, 10 male and 10 female individuals of both *C. frigida* and *C. pilipes* (n = 40) were added. All mesocosms were transferred to a controlled environment cabinet and maintained at 20 °C ± 2 °C, with a relative humidity of 60 % and a photoperiod of 12 h.

To enumerate bacterial concentrations from each replicate mesocosm, 10 g of seaweed or 5 g of sand were added to 10 ml or 5 ml of sterile seawater, respectively, and then vortexed for 1 minute. Luminescence (relative light units (RLU)) of the seaweed or sand supernatant was immediately measured using a SystemSURE 18172 luminometer (Hygiena Int., Watford, UK) to quantify relative *E. coli* O157:H7 metabolic activity. The remaining supernatant was serially diluted using sterile seawater, plated onto either CT-SMAC or R2A plates, and incubated as described above. *E. coli* O157:H7 and THB in both seaweed and sand were measured in each replicate mesocosm on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 15, 23, 31, 43 and 56; mesocosms containing the flies were not sampled on days 1 and 2 in order to allow mating to occur.

2.5 Survival and activity of *E. coli* O157: H7 in *C. frigida* larvae and wrack habitat during larval development.

This mesocosm experiment aimed to quantify the influence of larval feeding and development on the persistence and activity of *E. coli* O157:H7 associated with larvae and their wrack habitat, and to examine the effect of the removal of the naturally occurring larval-associated microbiota on the ability of larvae to digest or inactivate *E. coli* O157:H7. Mesocosms (n = 160) consisted of 100 ml sterile plastic pots (Gosselin™, Fisher Scientific UK Ltd) containing 25 g of seaweed (approximately 4 cm depth) placed on 20 g of sand (approximately 1.5 cm), with lids comprised of paper towelling to allow gas exchange. Prior to placement within each treatment and control mesocosm, the seaweed was divided into 1 kg batches, and inoculated by homogenising it for 5 min in a stomacher bag with 200 ml of *E. coli* O157:H7 contaminated seawater (4.42×10^7 CFU ml^{-1}). A pair of unmated *C. frigida* female and male flies was introduced to each mesocosm and removed 4 days later. On day 5, the resulting larvae were removed from these mesocosms and treated in one of four ways, (i) untreated, (ii) surface sterilised, (iii) starved, or (iv) surface sterilised then starved. Untreated larvae and larvae subjected to surface sterilisation only were removed for 3 h before returning 10 to each mesocosm. Starvation involved moving larvae to an empty sterile

container for 24 h, before returning 10 to each mesocosm. Thus sampling of mesocosms containing starved larvae lagged behind other treatments and the controls by 24 h. The aim of surface sterilisation was to reduce the microbial communities on the larval surface (although not completely eliminate them) and involved immersion in a 19:1 PBS:Ethanol solution for 1 min followed by two rinses in sterile PBS. Weak disinfectant was used in place of a potentially more effective stronger concentration in order to avoid incidental gut sterilisation of larvae due to larval ingestion of the disinfectant during immersion.

There were two control treatments from which larvae were absent: 40 mesocosms contained seaweed inoculated with *E. coli* O157:H7 laid on top of sand, and 40 mesocosms contained seaweed and sand both pre-sterilised by autoclaving (121 °C for 15 mins), after which the seaweed was inoculated with *E. coli* O157:H7. Destructive sampling of 10 larvae, 10 g seaweed and 5 g sand from replicate mesocosms (n = 4) from treatments and controls began six days after initial inoculation of seaweed and continued for eight successive days. Larvae (and pupae) were handled with sterile forceps and ground in 2 ml PBS in a 1.5 ml Eppendorf tube for 30 s with a micro pestle (Anachem Ltd., Bedfordshire, UK). Luminescence of the supernatant was immediately measured, and the homogenate serially diluted and plated onto either CT-SMAC or R2 agar as described above. *E. coli* O157:H7 concentration and relative activity and THB concentration were also enumerated in both seaweed and sand samples as described above. Mesocosms were maintained at 25°C ± 2°C, a relative humidity of 60% and a photoperiod of 12 h.

2.6 Transmission of *E. coli* O157:H7 by female and male *C. frigida* flies.

Using mesocosms, vector competence of *C. frigida* flies for metabolically active *E. coli* O157:H7 was assessed by investigating the capacity of females and males to separately contaminate previously uncontaminated seaweed and sand. Mesocosms (n = 80) consisted of 100 ml sterile plastic pots (Gosselin™, Fisher Scientific UK Ltd) containing 25 g of seaweed (approximately 4 cm) placed on 20 g of sand (approximately 1.5 cm), with lids comprised of paper towelling. Prior to

placement within each mesocosm, the seaweed was divided into 1 kg batches, and inoculated with 200 ml of *E. coli* O157:H7 contaminated seawater (6.9 x 10^7 CFU ml^{-1}). Ten *C. frigida* flies were added to each mesocosm; 40 mesocosms contained female flies, and 40 mesocosms contained male flies. After 24 h, all female and male flies were moved to 80 new mesocosms that contained 25 g of uncontaminated seaweed (approximately 4 cm), placed on 20 g of sand (approximately 1.5 cm), with paper towelling lids. After a further 24 h, eight replicate mesocosms (four female, four male) containing transplanted flies were destructively sampled, with 10 flies, 10 g seaweed and 5 g sand sampled from each mesocosm on nine successive days. Each fly was anaesthetised by 10 s exposure to CO_2 gas, and ground in 2 ml PBS in a 1.5 ml Eppendorf tube for 30 s with a micro- pestle. Luminescence of each fly supernatant was immediately measured, and the concentrations of *E. coli* O157:H7 and THB in the remaining supernatant determined as described above. *E. coli* O157:H7 and THB were enumerated, and relative activity measured, in the seaweed and sand as described above. All mesocosms were maintained at 25 °C ± 2 °C, at a relative humidity of 60 % and a photoperiod of 12 h.

2.7 Contribution of faecal excretion to transmission of *E. coli* O157:H7 by female and male *C. frigida* flies..

The potential for adult *C. frigida* faeces to facilitate the transmission and survival of *E. coli* O157:H7 was determined in mated females (*n* = 240), virgin females (*n* = 240) and virgin males (*n* = 240) in microcosms taking the form of individually enclosed Petri dishes (diameter 55 mm). Females were mated two days prior to the beginning of the experiment, and flies for all treatments were starved for 24 h before sampling began. Each Petri dish contained a sterile Eppendorf tube lid containing a feeding solution made from the liquid from decaying *L. digitata*. Half of the Petri dishes for each treatment (*n* = 120) contained feeding solution that had been contaminated by 250 µl of *E. coli* O157:H7 (1.09 x 10^2 CFU μl^{-1}), whilst the control groups (*n* = 120) received 250 µl of feeding solution uncontaminated with *E. coli* O157:H7. Previous observations determined that most

seaweed flies typically began producing faecal droplets 6 h after introduction of the feeding solution, and that fly mortality began after 24 h. Thus, the fly from each experimental and control mesocosm (n = 40 for each treatment) were sampled at 6 h, 12 h and 24 h, and *E. coli* O157:H7 concentration and relative activity measured in each individual fly, and in fly faeces. Faecal droplets belonging to each individual fly were counted, and a sterile toothpick used to transfer faeces from individual flies to 5 ml of LB Broth. Faeces were enriched overnight for 18 h at 100 rpm at 37 °C; cells were centrifuged, washed three times and re-suspended in PBS. Luminescence was quantified, and the solution serially diluted and plated onto CT-SMAC media to enumerate *E. coli* O157:H7 concentrations. Petri dish microcosms were maintained at 25 °C ± 2 °C, at a relative humidity of 60 % and a photoperiod of 12 h.

2.8 Statistical analysis

Data were normally distributed following $\log_{10}$ transformation, and analysis of variance (ANOVA) was applied to the data (SPSS 24.0 software, SPSS Inc. Chicago, IL, USA). A repeated measures (rm) ANOVA was used to test the effect of Coelopidae presence on *E. coli* O157:H7 concentration and relative activity in seaweed and sand, and a factorial ANOVA followed by Tukey post-hoc tests used to analyse the effect of larval feeding on *E. coli* O157 concentration, vector competency and the capacity of seaweed flies for biological transmission. Differences were considered significant at the $P \leq 0.05$ level.

3. RESULTS

3.1 Persistence and activity of *E. coli* O157:H7 in the presence of Coelopidae colonies.

The presence of *C. frigida* and *C. pilipes* flies significantly enhanced survival of *E. coli* O157:H7 attached to seaweed ($P < 0.001$) (Fig. 1a), and in the underlying sand ($P < 0.05$) (Fig. 1c), compared to mesocosms where flies were absent. Regardless of the presence or absence of flies, the concentration of *E. coli* O157:H7 peaked significantly in seaweed between days 9 and 15, and in sand

on day 11 ($P < 0.05$). Subsequent *E. coli* O157:H7 die-off to day 23 in seaweed was rapid in both treatments, reaching a concentration ~1 log CFU g^{-1} lower in the absence of flies than in seaweed associated with flies ($P < 0.05$). *E. coli* O157:H7 levels in sand were ~1 log CFU g^{-1} higher in the presence of flies than in the absence of flies between days 11 and 23 ($P < 0.05$), but the rate *E. coli* O157:H7 die-off in sand over two months was not significantly different between treatments. *E. coli* O157:H7 remained detectable in both seaweed and sand up to day 56. The presence of flies exerted no influence on the luminescence of *E. coli* O157:H7 in seaweed or sand (Fig. 1b and d).

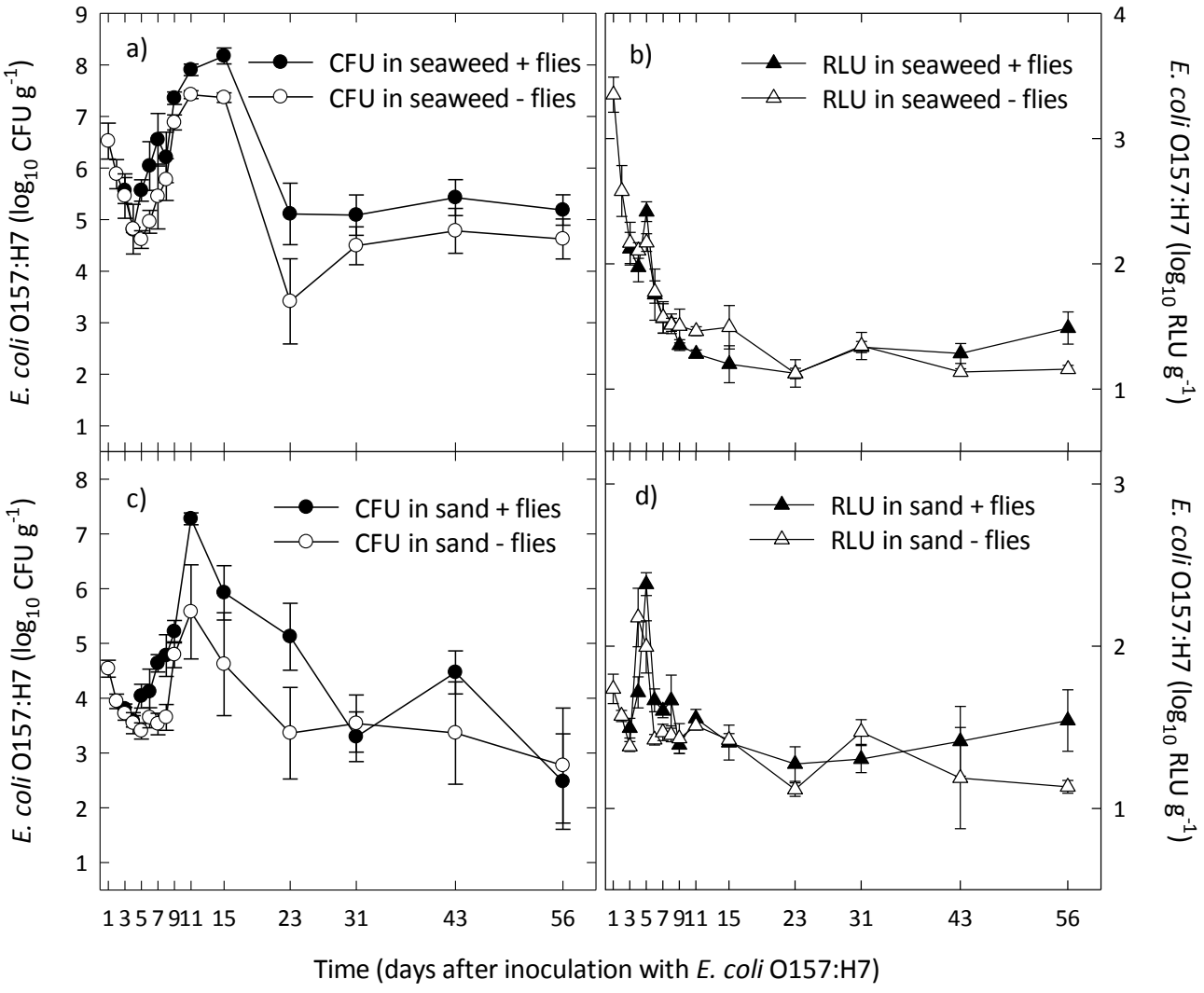


Fig. 1 Concentration in CFU (circles) in seaweed (a) and luminescence in RLU (triangles) in seaweed (b) of *E. coli* O157:H7 in mesocosms containing either flies (filled symbols) or no flies (open symbols). Concentration in CFU (circles) in sand (c) and luminescence in RLU (triangles) in sand (d) of *E. coli* O157:H7 in mesocosms containing either flies (filled symbols) or no flies (open symbols). Each mesocosm contained equal numbers of both *C. frigida* and *C. pilipes* flies. Data points represent the means ± SE

3.2 Survival and activity of *E. coli* O157: H7 in *C. frigida* larvae and wrack habitat during larval development.

By day 7, *E. coli* O157:H7 concentrations associated with untreated larvae fell to ~1 log CFU below that associated with sterilised, and starved and sterilised, larvae ($P < 0.05$), and by day 8 was ~1.5 log CFU lower than levels detected in all treated larvae ($P < 0.05$) (Fig. 2a). Reduction of the gut microbiota of larvae due to 24 h starvation led to significantly higher levels (~2 log CFU) of *E. coli* O157:H7 associated with starved larvae compared with untreated and surface sterilised larvae on day 4 ($P < 0.05$). Luminescence of *E. coli* O157:H7 increased significantly in untreated and sterilised larvae between days 2 and 3, and in starved, and starved and sterilised larvae, between days 3 and 4 ($P < 0.001$) (Fig. 2b). Luminescence of *E. coli* O157:H7 associated with untreated larvae exceeded that of all larvae that had reduced gut microbiota on days 3, 4, 7 and 8 ($P < 0.05$), and of starved larvae on day 5 ($P < 0.05$).

The presence of larvae suppressed *E. coli* O157:H7 concentrations in seaweed, compared with the non-sterile larvae-free mesocosms in which the concentration of *E. coli* O157:H7 associated with the seaweed increased over 8 days to 2 – 3 log CFU g^{-1} higher than all mesocosms containing larvae ($P < 0.001$) (Fig. 2c). Concentrations of *E. coli* O157:H7 in seaweed associated with untreated larvae increased rapidly by ~2 log CFU g^{-1} between days 5 and 6 ($P < 0.05$), whereas no significant change over time was observed in *E. coli* O157:H7 levels in seaweed associated with treated larvae. The luminescence of *E. coli* O157:H7 associated with seaweed in the mesocosms containing larvae and in the larvae-free mesocosms increased significantly between days 2 and 3 ($P < 0.05$), before levelling off (Fig. 2d). Luminescence in seaweed in the mesocosms containing untreated larvae was

375 significantly higher from day 3 onwards ($P < 0.05$), than in seaweed associated with larvae where the
376 gut microbiota had been reduced. However, the absence of natural seaweed microflora, and the
377 absence of larvae did not affect *E. coli* O157:H7 luminescence in seaweed. *E. coli* O157:H7 levels in
378 sand were not influenced by the presence or absence of larvae (Fig. 2e); however, from day 7 the
379 concentration of *E. coli* O157:H7 in the sand of mesocosms that contained either larvae that had
380 been both sterilised and starved, or the non-sterile mesocosms that contained no larvae, were
381 significantly higher than in the sand of mesocosms that contained either the surface-sterilised larvae
382 or the starved larvae ($P < 0.05$). The luminescence of *E. coli* O157:H7 in sand associated with larvae
383 and in larvae-free controls increased significantly between day 2 and 3 ($P < 0.05$), although there
384 was no significant difference between mesocosms that contained larvae and those that contained
385 no larvae (Fig. 2f).
386 In the absence of larvae and natural seaweed microflora, *E. coli* O157:H7 in seaweed and
387 sand of the sterile control mesocosms significantly exceeded levels in seaweed and sand in all
388 treatments containing larvae and of the non-sterile control on day 1 ($P < 0.001$). By day 8,
389 subsequent die-off of *E. coli* O157:H7 in seaweed in the sterile control mesocosms resulted in the
390 concentration being significantly lower than that of seaweed in the non-sterile control mesocosm (P
391 < 0.001), whilst *E. coli* O157:H7 concentration in sand by day 8 was no different to that in any of the
392 treatment or non-sterile control mesocosms. Luminescence of *E. coli* O157:H7 in sand was
393 significantly enhanced by the absence of natural microflora in the sterile control compared with the
394 non-sterile control on days 1, 2 and 4 ($P < 0.05$).
395

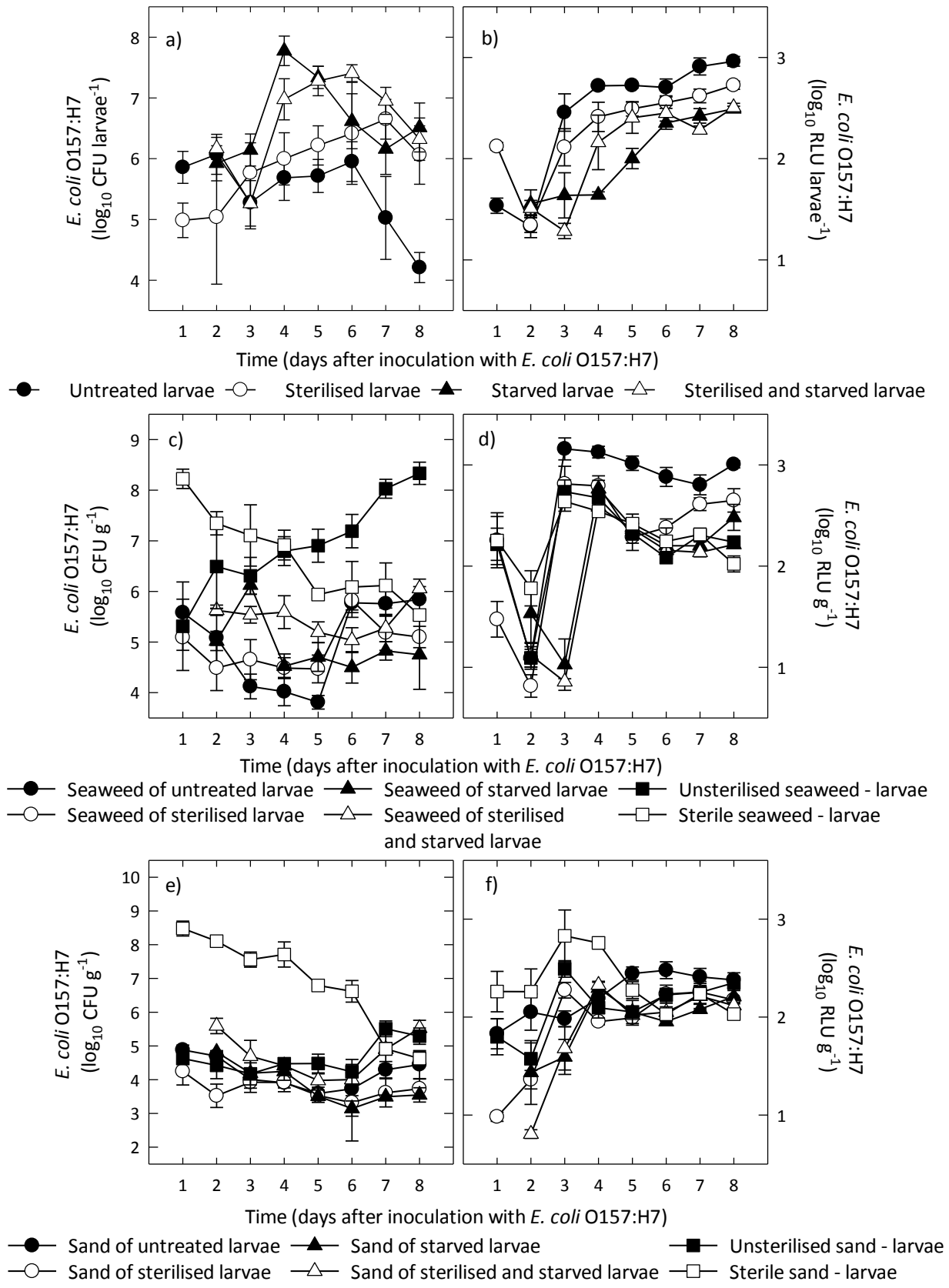
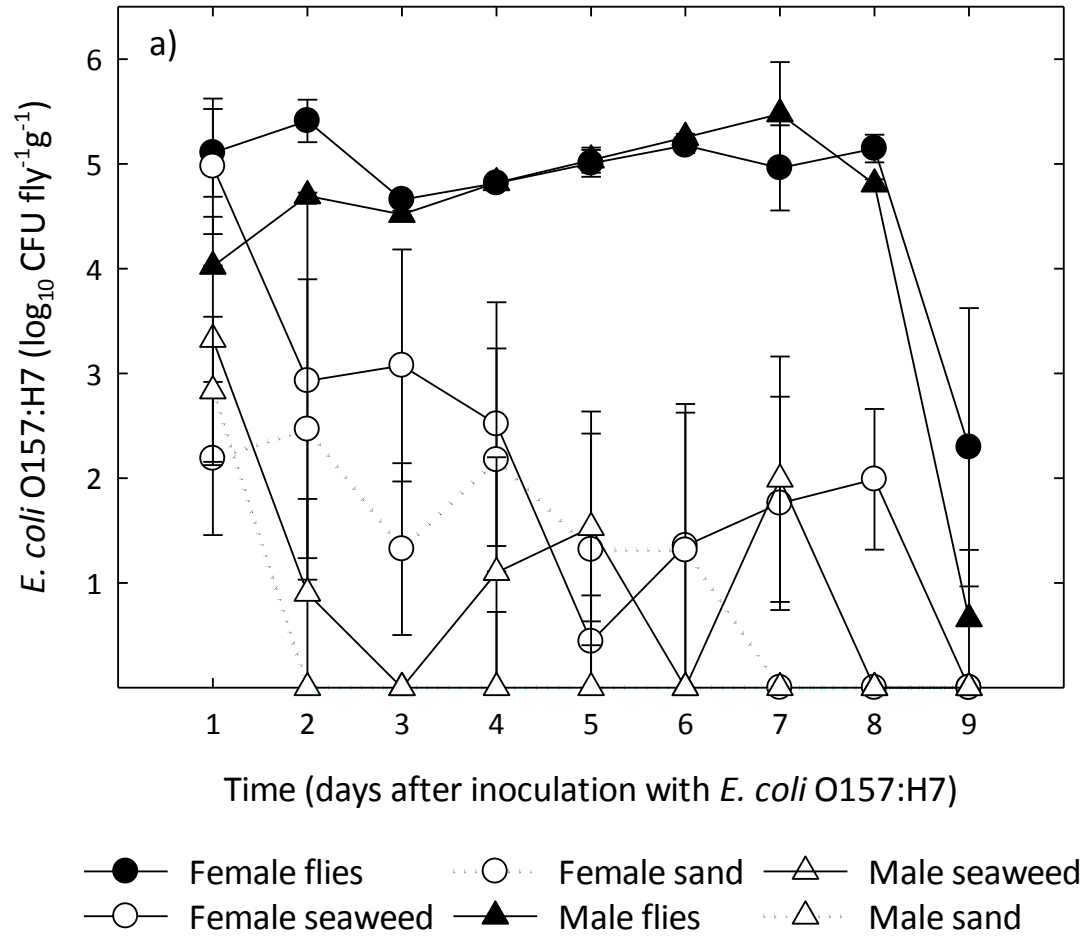


Fig. 2 Concentration in CFU (a, c and e) and luminescence in RLU (b, d and f) of *E. coli* O157:H7 in *C. frigida* larvae (a – b), seaweed (c - d) and sand (e - f) in mesocosms containing either untreated

larvae (filled circles), surface sterilised larvae (open circles), starved larvae (filled triangles) or sterilised and starved larvae (open triangles). Each mesocosm contained equal numbers of larvae. Control mesocosms without larvae contained either unsterilized substrate (filled squares) or sterilised substrate (open squares). Data points represent the means ± SE

3.3 Transmission of *E. coli* O157:H7 by female and male *C. frigida* flies.

Female and male *C. frigida* flies transmitted *E. coli* O157:H7 from contaminated seaweed to mesocosms previously free of the pathogen at concentrations > ~3 log CFU g^{-1} to seaweed and > ~2 log CFU g^{-1} to sand (Fig. 3a). The sex of the fly made no significant difference to the concentration of *E. coli* O157:H7 associated with the flies following 24 h exposure to contaminated seaweed, or on the subsequent persistence of the pathogen in flies until day 8. However, the gender of the flies in the mesocosms significantly influenced *E. coli* O157:H7 concentrations in seaweed on day 3, at which time pathogen levels on seaweed in female mesocosms were ~3 log CFU g^{-1} higher than on seaweed in male mesocosms ($P < 0.05$). *E. coli* O157:H7 concentrations in sand in female mesocosms significantly exceeded that of sand in male mesocosms by ~2.5 log CFU g^{-1} on day 2 ($P < 0.05$). Luminescence of *E. coli* O157:H7 in female flies, and the seaweed and sand in their mesocosms, was significantly higher than in male flies, seaweed and sand ($P < 0.001$) and increased significantly over time in both female and male flies ($P < 0.001$) (Fig. 3b). Maximum levels of *E. coli* O157:H7 luminescence occurred in flies, seaweed and sand on days 8, 8 and 4 respectively in female mesocosms and on days 8, 6 and 6 respectively in male mesocosms, significantly exceeding luminescence levels recorded in flies, seaweed and sand at all preceding and subsequent sampling points ($P < 0.05$).


a)
E. coli O157:H7 (log10 CFU fly-1g-1)
Time (days after inoculation with E. coli O157:H7)
Female flies
Female sand
Male seaweed
Female seaweed
Male flies
Male sand

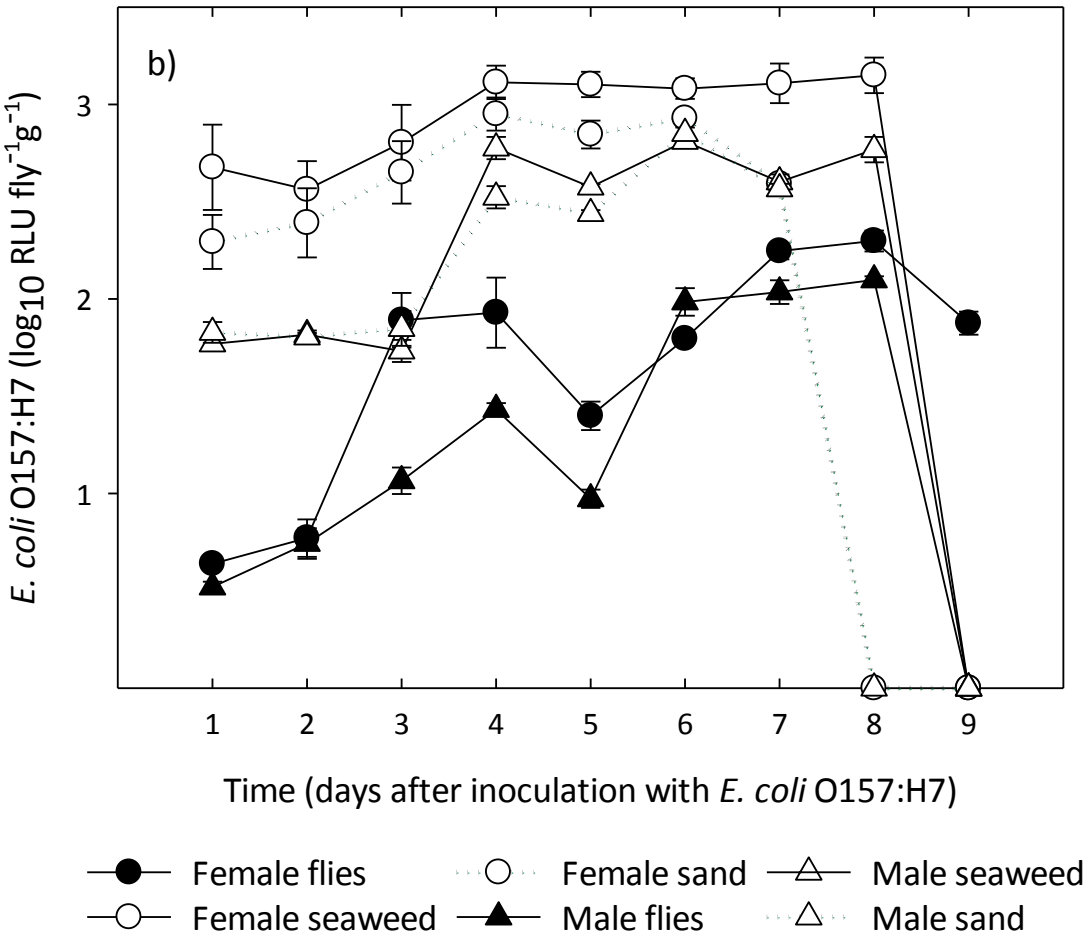

b)
E. coli O157:H7 (log10 RLU fly-1g-1)
Time (days after inoculation with E. coli O157:H7)
Female flies
Female sand
Male seaweed
Female seaweed
Male flies
Male sand

Fig. 3 Concentration in CFU (a) and luminescence in RLU (b) of *E. coli* O157:H7 in female flies (filled circles), male flies (filled triangles), seaweed (open circles) and sand (open circles with dotted line) in female *C. frigida* mesocosms, and seaweed (open triangles) and sand (open triangles with dotted line) in male *C. frigida* fly mesocosms. Data points represent the means ± SE

3.4 Contribution of faecal excretion to transmission of *E. coli* O157:H7 by female and male *C. frigida* flies..

After 6 h exposure to contaminated feeding solution, mated female flies were contaminated with significantly (~1 log CFU fly^{-1}) more *E. coli* O157:H7 than either virgin females or males ($P < 0.05$) (Fig. 4). By 12 h *E. coli* O157:H7 contamination had decreased in both females and males, with a significant reduction in mated females between 6 h and 12 h ($P < 0.001$). After 24 h exposure to *E. coli* O157:H7, 40 % of males, 7.5 % of mated females and 5 % of virgin females had died. The further decrease in contamination by *E. coli* O157:H7 between 12 h and 24 h was not significantly different between mated females and virgin males. However, between 12 h and 24 h *E. coli* O157:H7 concentrations in virgin females increased by ~1 log CFU fly^{-1}, and the final concentration at 24 h was significantly higher than levels associated with mated females and virgin males ($P < 0.05$). *E. coli* O157:H7 luminescence did not change significantly in female or male flies over 24 h, and there was no significant difference between mated and virgin females at 6 h, 12 h or 24 h; however, the luminescence of *E. coli* O157:H7 associated with males was consistently lower than in either female fly group ($P < 0.05$).

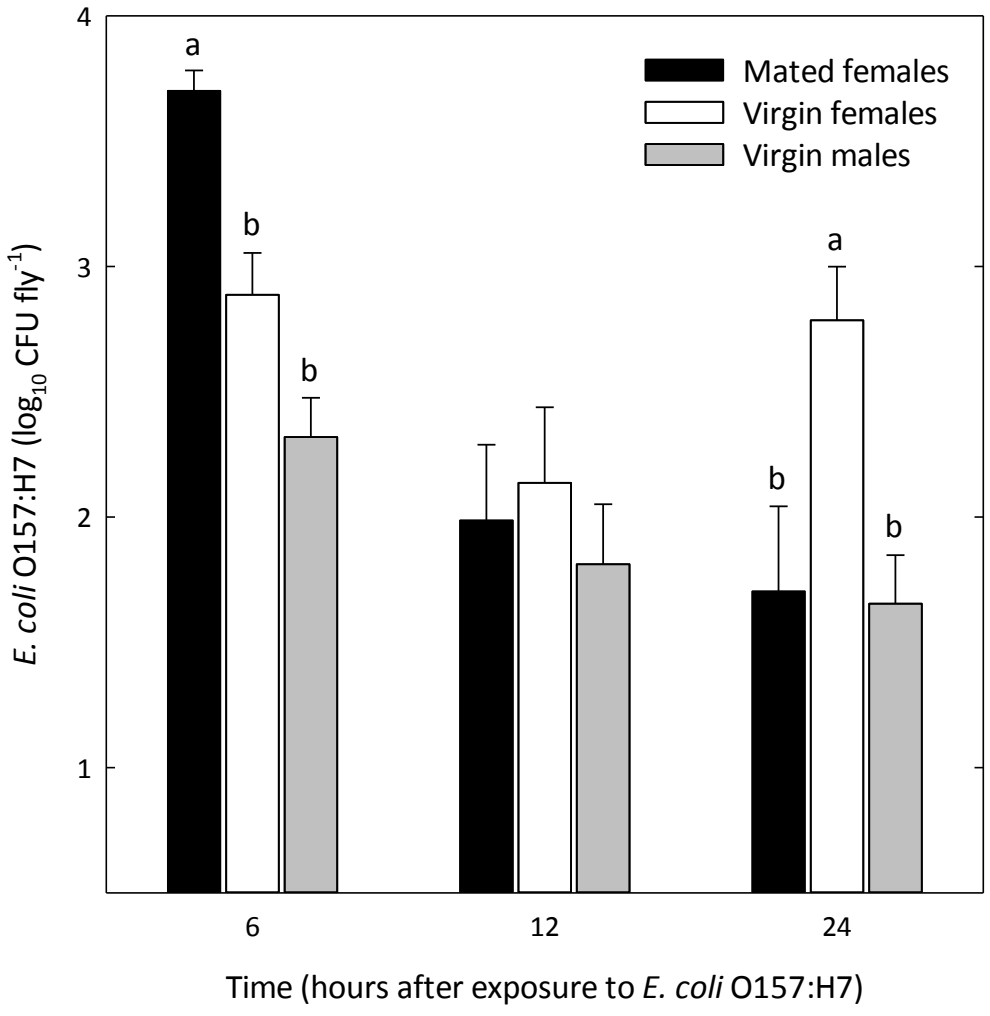


Fig. 4 *E. coli* O157:H7 concentrations in mated female flies (black bars), virgin female flies (white bars) and virgin male flies (grey bars) following exposure to inoculated feeding liquid. At each time point, bars that do not share a letter are significantly different from each other (two-way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$). Values represent the means +SE

Ingestion of *E. coli* O157:H7 had no effect on excretion levels in mated females, virgin females or virgin males relative to controls, and the number of faecal excretions produced by all flies in all mesocosms significantly increased between 6 h and 12 h, and again between 12 h and 24 h ($P < 0.05$). Faecal biomass and excretion rate were unaffected by the extent of egg maturation in females, but significantly exceeded that of males over the entire 24 h period ($P < 0.05$). The *E. coli* O157:H7 load in fly faeces increased significantly between 6 h and 12 h in females and males by ~2-

456 log CFU fly^{-1} ($P < 0.001$) followed by a significant reduction of 3 - 4 log CFU fly^{-1} in females and 2- log
457 CFU fly^{-1} in males ($P > 0.001$) by 24 h (Fig. 5). The sex of the fly affected the concentration of *E. coli*
458 O157:H7 in faecal excretions, with females producing ~4 log CFU fly^{-1} more of the pathogen in their
459 faeces than males at 6 h and 12 h, and ~2 - 3 log CFU fly^{-1} more than males at 24 h ($P < 0.001$). The
460 extent of egg maturation in females also affected levels of the pathogen in female faeces after 24 h
461 exposure, with concentrations in mated females being ~1 log CFU fly^{-1} greater than in virgin females
462 ($P < 0.05$). Luminescence of *E. coli* O157:H7 in the faeces of both female and male flies peaked at 12
463 h, increasing significantly between 6h and 12 h in male faeces and falling significantly between 12 h
464 and 24 h in virgin female faeces ($P < 0.05$). However, luminescence of *E. coli* O157:H7 in faeces did
465 not differ significantly between mated and virgin females during the 24 h; luminescence in faeces
466 from male flies remained significantly lower by comparison at all sampling times ($P < 0.001$).
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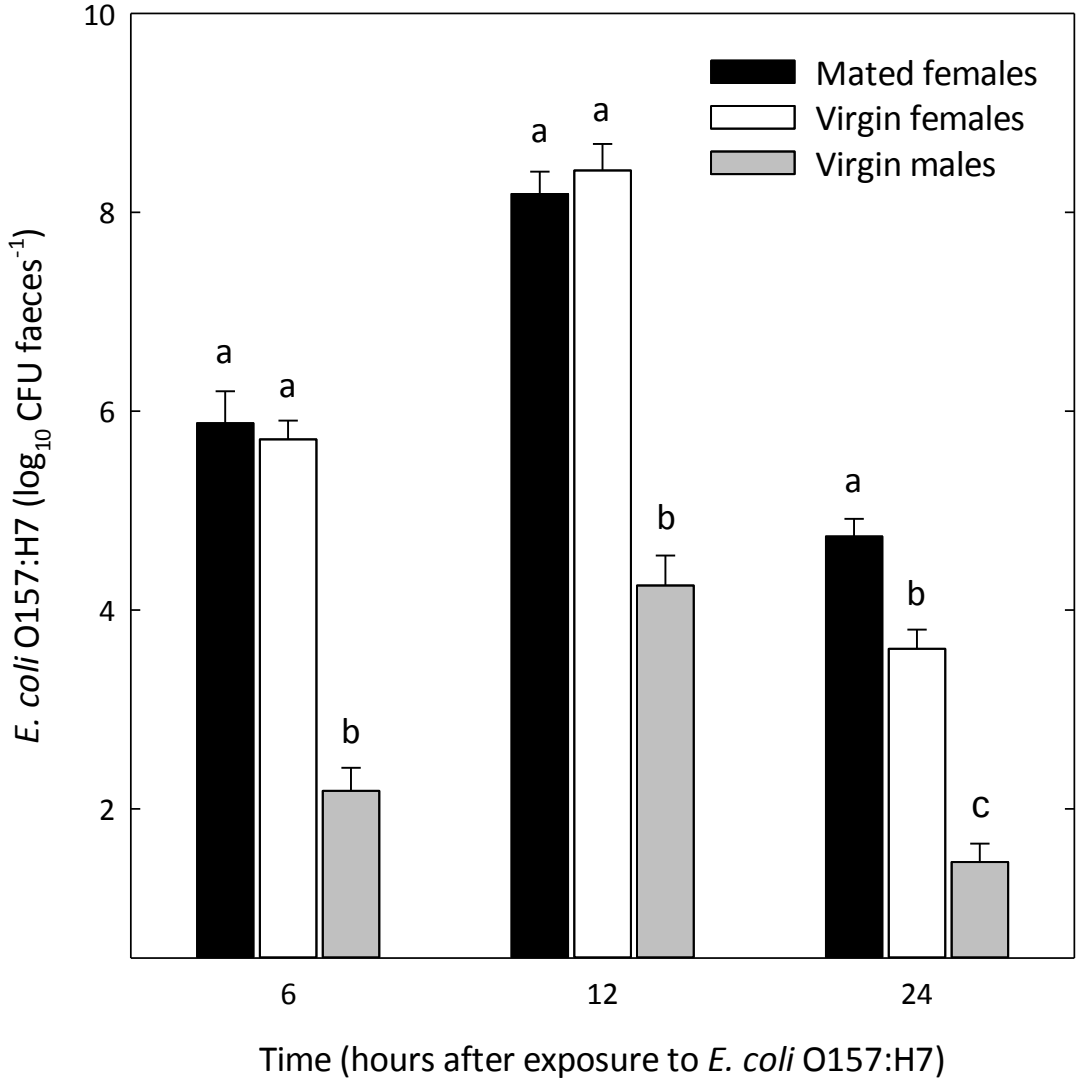

Fig. 5 *E. coli* O157:H7 concentration in the faeces of mated female flies (black bars), virgin female flies (white bars) and virgin male flies (grey bars) following exposure to inoculated feeding liquid. At each time point, bars that do not share a letter are significantly different to each other (two-way ANOVA, $P< 0.05$; Tukey's test, $P< 0.05$). Values represent the means +SE

4. DISCUSSION

The role of non-synanthropic Diptera in the environmental dissemination of human pathogenic bacteria has not been previously examined within a public health context. This study has demonstrated that an endemic species of seaweed fly (*C. frigida*) commonly found in dense populations on public beaches throughout Europe is capable of facilitating the dispersal of *E. coli*

O157:H7 between individual seaweed habitats, and further transmission to beach sand. An important mechanism for this transmission is in faecal excretions by adult flies. Furthermore, the presence of both *C. frigida* and *C. pilipes* enhanced growth of *E. coli* O157:H7 in simulated wrack bed environments, and in the underlying sand. Activity by multiple generations of flies and larvae in decaying wrack beds modifies their habitat by altering the physio-chemical composition of the substrate, and can facilitate microbial growth and persistence [Cullen et. al., 1987]. This study provides the first evidence that seaweed fly populations inhabiting natural wrack beds contaminated with the human pathogen *E. coli* O157:H7 have the capacity to amplify the hazard source, and therefore potential transmission risk, to beach users exposed to seaweed and sand in the intertidal zone.

Following ingestion of high concentrations of *E. coli* O157:H7, seaweed flies were rapidly internally contaminated with the pathogen and within 6 h produced faeces containing viable (metabolically active) *E. coli* O157:H7 bacteria at concentrations exceeding the infectious dose for humans [Teunis et. al., 2004]. Homogenisation of whole flies in order to measure individual infection with *E. coli* O157:H7 prevented evaluation of the separate contributions of bacterial attachment to exoskeletons compared with ingestion to overall individual contamination. However, a degree of external carriage of the pathogen was highly probable as flies of both sexes were observed in and on feeding solution dishes. Therefore, greater carriage of *E. coli* O157:H7 after 6 h by mated females than virgin females, and *vice versa* at 24 h, cannot be solely attributed to differences in ingestion volume or rate, possibly due to stage of egg development [Sasaki et. al., 2000]. However, greater contamination of mated female flies than males at 6 h, and of virgin females than males at 24 h may indicate that reproductive biology influences ingestion volume and rate, most likely due to physiological requirements associated with egg production.

A distinction should be drawn between studies in which fly exposure to a pathogen is via a single food droplet, and those that allow constant feeding as in the present study, which more realistically reflects the availability of the source in natural wrack beds. The former might be

508 expected to produce a steady decline over time in pathogen concentration in flies due to clearance
509 from their digestive tracts, whereas the latter may generate a more variable result due to multiple
510 feeding opportunities [Fleming et. al., 2014]. It is possible that the higher levels of activity of mated
511 and virgin female compared with males in the Petri dish microcosms may have resulted in additional
512 contamination of the female exoskeleton leading to higher overall *E. coli* O157:H7 concentrations on
513 mated females at 6 h and virgin females at 24 h. Importantly, the concentration of *E. coli* O157:H7
514 associated with the flies did not consistently increase during exposure to the contaminated feeding
515 solution; seaweed flies are therefore unlikely to be reservoirs of *E. coli* O157:H7, meaning that the
516 pathogen load associated with individuals is dependent upon levels of contamination in seaweed,
517 and is not influenced by disease maintenance amongst seaweed fly populations [Caron et. al., 2015].
518 The lower luminescence, and hence, relative metabolic activity, of *E. coli* O157:H7 associated
519 with male flies compared with females over 24 h may indicate that efficacy of inactivation of the
520 pathogen in the seaweed fly gut is partly related to the sex of the fly. This is unlikely however, given
521 that in Dipteran digestive tracts the efficacy of antibacterial effectors active against non-native gut
522 bacteria (the innate response) depends primarily on the species of fly and the vulnerability of the
523 bacterial species to that response [Nayduch and Burrus, 2017]. The fate of ingested *E. coli* O157:H7
524 may also be dose-dependent, meaning that below or above a certain dose threshold, bactericidal
525 substances in seaweed fly digestive tracts may be effective against ingested cells of the pathogen
526 [Kumar and Nayduch, 2016]. It is likely that external *E. coli* O157:H7 contamination of both mated
527 and virgin female seaweed flies will be greater than males due to their higher physical activity. By
528 contrast, if the majority of male contamination was internal and thus vulnerable to gut inactivation,
529 this might account for the consistently lower metabolic activity of *E. coli* O157:H7 associated with
530 male flies compared with females.
531 The mechanisms of bacterial transmission by flies to various surfaces via regurgitation and
532 faecal excretion are well established [Pava-Ripoll et. al., 2012], and passage of *E. coli* O157:H7
533 through the digestive tract of seaweed flies did not entirely inactivate this pathogen. Female *C.*

534 *frigida* produced more faecal excretions on average than male *C. frigida*, suggesting a more rapid
535 ingestion rate by females than males, which was also matched by a faster excretion rate. Clearance
536 of *E. coli* O157:H7 from the digestive tracts of female *C. frigida* was more rapid than their ingestion
537 rate, whereas males excreted *E. coli* O157:H7 at approximately the same rate as they ingested the
538 pathogen. The excretion rate by both female and male flies approximately doubled between 6 - 12
539 h, and 12 - 24 h, although this was not mirrored by the concentration of faecal *E. coli* O157:H7, most
540 likely due to the rate of pathogen die-off in the feeding solution. The concentration of *E. coli*
541 O157:H7 in the faeces of both sexes may be underestimated due to desiccation of most excreta by
542 12 h which would have affected recovery, and the decreasing availability of the feeding solution due
543 to evaporation over 24 h. However, these results do demonstrate that at 12 h after initial ingestion
544 of *E. coli* O157:H7, both female and male seaweed flies present the greatest risk of pathogen
545 transmission via faecal excretion.

546 Female and male seaweed flies were capable of vectoring *E. coli* O157:H7 to seaweed and
547 sand 24 hours after exposure to the pathogen. The faster rate of faecal production by female *C.*
548 *frigida*, and thus greater quantity of excretion of *E. coli* O157:H7 compared with males, represents
549 the underlying mechanism for the greater pathogen load transmitted by females than males to
550 simulated wrack habitats. Excretion droplets have been shown to be 'hotspots' of *E. coli* O157:H7
551 when the pathogen was fed to houseflies [Sasaki et. al., 2000], and viable populations of this
552 pathogen remained in seaweed fly faeces for at least 24 h after initiation of feeding on *E. coli*
553 O157:H7. The persistence of *E. coli* O157:H7 on seaweed and sand demonstrates that seaweed fly
554 excretions onto the surface of wrack and sand provided favourable conditions for *E. coli* O157:H7
555 persistence in these substrates. Survival of the pathogen in and on the flies is thus maintained by
556 continual ingestion and recontamination of the exoskeleton from the wrack habitat.

557 Temperature is a key determinant of the distribution of the cold-favouring *C. frigida* and a
558 northward shift in their northern European range in recent decades is a likely response, in part, to a
559 simultaneous warming trend in this region [Phillips et. al., 1995, Edward et. al., 2007, IPCC, 2013].

Mass migration of *C. frigida* adults over considerable distances has been reported, included nuisance inland plagues in urban areas, possibly driven by sub-optimal habitat conditions, or alternatively optimal conditions supporting high population densities [Egglishaw, 1961, Oldroyd, 1954]. The phenomenon of inland emigration of seaweed flies indicates that the presence of decaying seaweed is not a pre-requisite attractant for their dispersal, although the absence of wrack habitat inland would prevent establishment of a population in that location. Female *C. frigida* can lay three clutches of up to 80 eggs each and in mainland Europe this species is normally more abundant, and experiences a faster egg to adult development time, than *C. pilipes* which lay single eggs [Dobson 1974a and 1974b, Edward et. al., 2007]. *C. frigida* larvae typically occur at densities of approximately 1000 larvae kg^{-1} of seaweed, and in optimal conditions, *C. frigida* populations have the potential to increase by approximately 200 times with each generation [Butlin et. al., 1984, Dobson, 1974a]. Thus, the potential for *E. coli* O157:H7 transmission by migrating female and male *C. frigida* within and between beaches, and even inland, should not be underestimated. The ability of seaweed flies to vector *E. coli* O157:H7 from contaminated wrack beds on beaches to recently deposited seaweed, together with intraspecific transmission to other seaweed fly populations, therefore increases the spatial reach of the risk of public exposure to this pathogen.

Persistence and growth of *E. coli* O157:H7 in seaweed and sand both in the presence and absence of seaweed flies confirms that the simulated wrack environment facilitates long term survival of *E. coli* O157:H7. Both seaweed and sand provide a source of environmental exposure to the pathogen, which ensure that several generations of *C. frigida* and *C. pilipes* flies are continually externally and internally contaminated and re-contaminated [Graczyk et. al., 2001]. Thus, a single wrack bed could ensure the persistence of *E. coli* O157:H7 and subsequent vectoring by several generations of seaweed flies; however, wrack beds in the natural environment are transient habitats, often present for no more than a few days [Edward et. al., 2007]. Furthermore, laboratory conditions protected *E. coli* O157:H7 from predation, UV radiation, and provided plentiful nutrients, water and a favourable temperature [O'Mullan et. al., 2017]. Therefore, depending on vulnerability

586 to high tides and internal wrack bed temperatures attained, the observed growth of *E. coli* O157:H7
587 in seaweed and sand from day 4 may occur only sporadically in beach environments, meaning that
588 production of a single cohort of *E. coli* O157:H7 contaminated seaweed flies from a single wrack bed
589 is more likely than production of multiple cohorts. Additionally, the predominance of a single
590 bacterial species in the larval diets, and presence of two seaweed species only, contrasts with the
591 diverse microbial assemblage associated with the multiple seaweed species present in natural wrack
592 beds [Edward et. al., 2008]. Restriction to a sub-optimal diet, however, affected all treatments
593 equally, and seaweed fly larvae have been shown to survive on a monospecific diet of commensal *E.*
594 *coli*, suggesting that feeding and development were not greatly impaired by these experimental
595 conditions [Cullen et. al., 1987].

596 Interestingly, *C. frigida* adult flies facilitated the survival of *E. coli* O157:H7 in wrack bed
597 habitats over 56 days due to excretion of viable cells of the pathogen following ingestion, despite the
598 presence of multiple generations of larvae, whilst *C. frigida* larvae alone initially suppressed
599 populations of *E. coli* O157:H7 in the seaweed they inhabited. The onset of pupation on
600 approximately day 6 coincided with reductions in the concentration of *E. coli* O157:H7 in larvae and
601 pupae. This phenomenon, recorded for other species of fly larvae and human pathogens, could be
602 caused by cessation of feeding and subsequent voiding of digestive tracts prior to pupation, and the
603 destruction or inactivation of *E. coli* O157:H7 by gut microbes during metamorphosis [Lalander et.
604 al., 2013, Engel and Moran, 2013]. The efficiency by which these mechanisms reduced pathogen
605 loads in seaweed fly larvae, and resulted in increased *E. coli* O157:H7 on seaweed was dependent on
606 larvae possessing a full complement of native gut microbiota. Examination of the possible
607 contribution of loss of surface microbiota from seaweed fly larvae to reduction of *E. coli* O157:H7
608 concentrations within the larvae may have been confounded by ineffective surface disinfection of
609 larvae. This may have contributed to the lack of distinction between detected pathogen loads in
610 untreated and surface sterilised larvae throughout most of the sampling period. In the absence of
611 seaweed fly larvae and a diverse community of competing microbiota in seaweed and sand, the

612 initial rapid growth of *E. coli* O157:H7 was not sustained. In a contamination scenario of decaying
613 wrack beds contaminated with lower concentrations of *E. coli* O157:H7, seaweed fly larvae may be
614 capable of greater levels of pathogen reduction in seaweed and in the underlying sand. However,
615 concentrations of *E. coli* O157:H7 in or on larvae and pupae may still exceed that of their substrate
616 during their development, and at any level of contamination may be capable of passive transmission
617 of the pathogen between wrack habitats given that larvae washed by the sea from wrack beds can
618 survive 48 h of immersion in seawater [Dobson, 1974a]. Further research is required to determine if
619 *E. coli* O157:H7 can be transmitted trans-stadially between seaweed fly pupae and newly eclosed
620 adult flies, and at what concentration might *E. coli* O157:H7 ingested by larvae produce adults
621 immediately capable of vectoring the pathogen between wrack habitats [Schuster et. al., 2013].
622 The typical management response at popular recreational sandy beaches is to remove
623 decaying seaweed, which also appeals to the public's aesthetic preferences [Quilliam et al., 2015],
624 yet this has been shown to elicit either no change or an increase in faecal indicator organisms, such
625 as *E. coli*, in nearshore water [Russell et. al., 2014]. In addition, wrack removal reduces richness of
626 invertebrate species inhabiting wrack beds, including *C. frigida* and *C. pilipes* [Gilburn, 2012].
627 Management of diffuse and point sources of *E. coli* O157:H7 in the environment can help to reduce
628 *E. coli* O157:H7 inputs into beach environments, and farm-level strategies to reduce direct
629 defecation by livestock and diffuse agricultural runoff to the coastal zone are important to mitigate
630 the transfer of pathogens and nutrients to coastal environments [Young, 2016]. Excessive nitrogen
631 loading of coastal waters is a major cause of accelerated seaweed production, resulting in
632 unnaturally high levels of wrack biomass accumulating along coastlines [Anderson et. al., 2002]. In
633 such a scenario, and in combination with warmer temperatures as a result of climate change, the
634 availability of seaweed biomass for attachment by human pathogens including *E. coli* O157:H7,
635 combined with the subsequent growth of seaweed fly populations due to increased habitat
636 availability, could potentially increase the opportunity for seaweed flies to function as bridge hosts
637 and disseminate human pathogens at recreational beaches.

CONCLUSION

Seaweed flies and their larvae form large natural populations in recreational beach environments and can act as bridge hosts of the human pathogen *E. coli* O157:H7. However, they are restricted to decaying wrack beds and their dispersal is limited to beach environments where that habitat occurs. Therefore, despite seaweed flies facilitating long-term survival of *E. coli* O157:H7 in seaweed and sand, and flies and larvae potentially disseminating the pathogen amongst individual wrack beds and seaweed fly populations, both vectors and reservoirs are spatially constrained within the environment. The risk to public health from seaweed flies and decaying wrack beds is usually limited by human avoidance behaviour. However, beach sand can act as a significant reservoir with which the public make far more deliberate contact, particularly following beach grooming and the removal of seaweed.

CONFLICT OF INTEREST

Declarations of interest: none.

COMPLIANCE WITH ETHICAL STANDARDS

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

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