1   **Systems-thinking approach to identify and assess feasibility of potential interventions to reduce**

2   **antibiotic use in tilapia farming in Egypt**

3   Andrew P. Desbois[1], Maria Garza[2], Mahmoud Eltholth[1,3,4], Yamen M. Hegazy[5], Ana Mateus[2], Alexandra

4   Adams[1], David C. Little[1], Erling Høg[6], Chadag Vishnumurthy Mohan[7], Shimaa E. Ali[7,8], Lucy A. Brunton[2*]

5   [1]Institute of Aquaculture, University of Stirling, FK9 4LA, United Kingdom

6   [2]Royal Veterinary College, University of London, AL9 7TA, United Kingdom

7   [3]Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University,

8   Kafrelsheikh, Egypt

9   [4]Global Academy of Agriculture and Food Security, The Royal (Dick) School of Veterinary Studies, University

10   of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, UK

11   [5]Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh,

12   Egypt

13   [6]Department of Global Health and Development, London School of Hygiene and Tropical Medicine

14   [7]WorldFish

15   [8]Department of Hydrobiology, National Research Centre, Egypt

16   *corresponding author: lbrunton@rvc.ac.uk +44 1707 666025

17   **Abstract**

18   Antibiotics are used in aquaculture to maintain the health and welfare of stocks; however, the emergence

19   and selection of antibiotic resistance in bacteria poses threats to humans, animals and the environment.

20   Mitigation of antibiotic resistance relies on understanding the flow of antibiotics, residues, resistant

21   bacteria and resistance genes through interconnecting systems, so that potential solutions can be identified

22   and issues around their implementation evaluated. Participatory systems-thinking can capture the deep

23   complexity of a system while integrating stakeholder perspectives. In this present study, such an approach

24   was applied to Nile tilapia (*Oreochromis niloticus*) production in the Nile Delta of Egypt, where disease

25   events caused by antibiotic-resistant pathogens have been reported. A system map was co-produced with

26   aquaculture stakeholders at a workshop in May 2018 and used to identify hotspots of antibiotic use,

27   exposure and fate and to describe approaches that would promote fish health and thus reduce antibiotic

28   use. Antibiotics are introduced into the aquaculture system via direct application for example in medicated

29   feed, but residues may also be introduced into the system through agricultural drainage water, which is the

30   primary source of water for most fish farms in Egypt. A follow-up survey of stakeholders assessed the

31   perceived feasibility, advantages and disadvantages of potential interventions. Interventions that

32   respondents felt could be implemented in the short-term to reduce antibiotic usage effectively included:

33   *more frequent water exchanges*, *regular monitoring of culture water quality parameters*, *improved storage*

34   *conditions for feed*, *use of probiotics* and *greater access to farmer and service providers training*

35   *programmes*. Other potential interventions included *greater access to suitable and rapid diagnostics*, *high*

36   *quality feeds*, *improved biosecurity measures* and *genetically-improved fish*, but these solutions were

37   expected to be achieved as long-term goals, with cost being of one of the noted barriers to

38   implementation. Identifying feasible and sustainable interventions that can be taken to reduce antibiotic

39   use, and understanding implementation barriers, are important for addressing antibiotic resistance and

40   ensuring the continued efficacy of antibiotics. This is vital to ensuring the productivity of the tilapia sector

41   in Egypt. The approach taken in the present study provides a means to identify points in the system where

42   the effectiveness of interventions can be evaluated and thus it may be applied to other food production

43   systems to combat the problem of antibiotic resistance.

44   **Keywords**

45   Antibiotic resistance; antimicrobial resistance; One Health; aquaculture; tilapia; Egypt

46

47

1. Introduction

Antibiotic resistance (ABR) is one of the greatest challenges we face in the 21st century and it is a classical One Health problem with human, animal and environmental components (Robinson et al., 2016). Antibiotic-resistant bacteria and antibiotic-resistance genes (ARG) transfer between these components, which complicates the tracking of their flow and adds complexity when developing solutions (Robinson et al., 2016). The problem of ABR must be addressed to maintain the effectiveness of antibiotics in food production and for patients in healthcare settings.

The global aquaculture sector is a major user of antibiotics, where these agents are applied to maintain the health and welfare of stocks, though usage and practices vary widely across the world (Henriksson et al., 2018; Lulijwa et al., 2020). Accordingly, the problems posed by ABR vary, though much of the burden of the issues encountered falls on low- and middle-income countries (LMICs), where controls on antibiotics may not be as strict or as strongly enforced as in wealthier counterparts (Robinson et al., 2016; Henriksson et al., 2018).

ABR in aquaculture poses a threat to human health and to the contamination of the environment with antibiotic residues and resistant organisms. Many of the classes of antibiotics used in aquaculture are identical to those used to treat terrestrial farm animals and human patients (Rico et al., 2013; Done et al., 2015; Lulijwa et al., 2020). In LMICs, aquaculture systems are highly complex and often integrated with other food production systems (Cantas et al., 2013; Chuah et al., 2016; Watts et al., 2017; Shen et al., 2018), such as through sharing of common water sources, making them highly vulnerable to the introduction and widespread dissemination of ABR (Cabello et al., 2016; Brunton et al., 2019). The complexity of these systems creates multiple points for human exposure to antibiotic residues, ARGs and resistant organisms; our earlier study identified three key pathways for human exposure to these to occur through occupational duties, consumption of contaminated food and environmental exposure (Brunton et al., 2019).

72 Egypt produces more fish from aquaculture than the rest of Africa combined, with production trebling since

73 2005 to 1.56 million tonnes in 2018 (FAO, 2020). Aquaculture in Egypt is dominated by the production of

74 Nile tilapia (*Oreochromis niloticus*) in earthen ponds, with production concentrated in the northern regions

75 of the Nile Delta. The sector has undergone considerable expansion in recent decades, driven by the

76 development of privately-owned hatcheries and feed mills (El-Sayed, 2017), and Egypt produced 1,051,444

77 tonnes of tilapia in 2018 (FAO, 2021b). The sector is composed mainly of small-scale producers and the

78 value chain, including production constraints and consumer behaviour and preferences, has been described

79 in detail recently (Eltholth et al., 2015). Like tilapia producers elsewhere, fish farmers in Egypt experience

80 disease challenges (Ali et al., 2020), including infectious diseases caused by bacteria such as *Aeromonas*

81 *hydrophila*, enterococci species, *Pseudomonas fluorescens* and *Streptococcus iniae* (Aly, 2013; Osman et al.,

82 2016; Osman et al., 2017). Antibiotics are used by farmers to treat infections in their stocks but antibiotic-

83 resistant pathogens have been reported to cause infections in farmed tilapia in Egypt (Eltholth et al., 2015;

84 Ishida et al., 2010; Osman et al., 2016; Osman et al., 2017). It is essential that this problem is addressed to

85 ensure the sustainability and continued productivity of the sector, which supports livelihoods, particularly

86 in rural communities, and is a major source of affordable animal protein for the country's population.

87 Indeed, almost all tilapia production in Egypt is for domestic consumption and only 2,059 tonnes of product

88 were exported in 2018 (FAO, 2021a).

89 Many approaches can help to mitigate against the problems posed by ABR and this includes interventions

90 that decrease overall use of antibiotics, such as by reducing need and dependency on them. When

91 identifying mitigation measures for ABR, including ways to reduce the need for their application, it is vital to

92 include all relevant stakeholders in the process and the decision-making. Such participatory approaches

93 capture the range of views, lead to a sense of collective responsibility towards the problem, and ensure a

94 feeling of shared ownership towards solutions (Evans and Terrey, 2016; Blomkamp, 2018). Systems thinking

95 attempts to integrate the multi-level aspects of a system, including actors, processes, and governance

96 structures. The approach can be used to generate a map that can then be used to understand how an

97 intervention in one part of the system to address a problem will impact on the functioning of the entire

98 system, thus permitting the recognition of consequences in an attempt to avoid unintended negative

99 outcomes elsewhere in the system (Peters, 2014). Brunton et al. (2019) used a participatory system-

100 thinking approach to consider points in aquaculture systems in Vietnam where ABR could emerge, or be

101 enriched through selection, by exposure to antibiotics and other selectors of ARGs.

102 In this present study, we aimed to identify potential interventions that may reduce antibiotic use (ABU) in

103 tilapia production systems in Egypt through a participatory systems-thinking approach with key

104 stakeholders. To meet this aim, it was necessary to (1) map the tilapia production system in Egypt; (2)

105 identify hotspots of ABU, exposure and fate within the system; (3) describe approaches that would

106 promote fish health, including the use of alternatives to antibiotics, and (4) conduct a follow-up survey of

107 key stakeholders to assess the perceived feasibility, advantages and disadvantages of potential

108 interventions to reduce or prevent ABU, including actual or perceived barriers to implementation.

109

110 **2. Methods**

111 *2.1. Stakeholder workshop*

112 A one-day participatory workshop was organised with aquaculture stakeholders in Kafrelsheikh, Egypt, on

113 10 May 2018. The workshop involved 44 participants from a range of disciplines and stakeholder groups,

114 including professionals from the aquaculture, livestock and veterinary sectors working in the private sector

115 (e.g., tilapia producers, input producers, agrovets, feed producers, and pharmaceuticals), academics, and

116 employees of international development and public sector institutions (Table 1). The expertise among

117 participants included aquaculture and aquatic health management, veterinary epidemiology and public

118 health, food safety, microbiology, marine biotechnology and medical anthropology. This range of

119 participants was invited to capture a diversity of perspectives, expertise and experiences, and to maximise

120 engagement and collaboration amongst distinct stakeholder groups. Participants were allocated to three

121 groups, each containing a mix of expertise. Outputs were compared between the groups, so to permit

122   cross-checking and discussion of the outputs as a method of validation. Each group contained two

123   members of the research team, one of whom facilitated the activity (the facilitator) and another who took

124   notes of discussions throughout the process (the recorder). All workshop activities were facilitated in

125   English and simultaneously translated into Arabic by members of the research team and participants,

126   where required.

127   The workshop activities consisted of introductory presentations, the main mapping exercise, and a final

128   plenary activity. During the introduction, participants were provided with a contextual overview of tilapia

129   production and value chain in Egypt, including post-harvest operations, the workshop aim and objectives

130   and planned activities, as well as an introduction to systems thinking and its application to food production

131   systems. To familiarise participants with the systems concept and the mapping process, an exercise was

132   conducted whereby each group was asked to draw a simple system on large whiteboards or paper sheets

133   affixed to the wall, exemplifying 'making a cup of tea'. Groups had to identify elements of the system such

134   as tangible and intangible components, including actors, infrastructure, governance systems and

135   relationships, and economic and environmental factors. The exercise introduced participants to the

136   mapping process and the importance of establishing boundaries (i.e., 'edges') with other systems. This

137   approach is based on the methodological framework proposed by the Network for the Evaluation of One

138   Health (NEOH) (Rüegg et al., 2018).

139   2.2. Mapping of the tilapia system

140   Each group was guided through the mapping activity to accomplish a series of objectives, and these

141   objectives were framed to consider four dimensions of the system (Figure 1). The groups were asked to:

142   • *Map* the components of the system (e.g. production stages, inputs, outputs, actors and

143   relationships);

144   • *Identify* any factors in the system that impact production and fish health;

145   • *Identify* where ABU and antibiotic residues occur in the system;

146   • *Explore* drivers of ABU in the system and decision making processes of stakeholders;

147   • *Discuss* possible interventions and alternatives to antibiotics, and

148   • *Identify* knowledge and data gaps.

149   The four dimensions within which these objectives were considered were the *human dimension*, related to

150   stakeholder behaviour; *the pathogen and host factors*, including information on common pathogens and

151   diseases in tilapia; *the environment*, considering information on related systems such as the agricultural

152   and drainage systems; and *governance*, considering the influence and power of different stakeholders on

153   decision-making or practices. A protocol was developed by the research team members to facilitate the

154   mapping process, which included the key elements to be captured, such as suggestions for alternatives to

155   antibiotics, and prompting questions to promote discussion and participation.

156   Participants were asked to identify hotspots on the maps where antibiotic residues or ARGs may be

157   present. Hotspots were defined in three ways, concerning system *nodes*, system *inputs* and *outputs*, and

158   system *management*. Nodes in the system represent junctions, where there is active use of antibiotics, or

159   where there is a potential presence of antibiotic residues or ARGs due to the flow of products through the

160   system (e.g., hatchery, nursery ponds, grow-out ponds, poultry farms, etc.). Products, inputs or outputs of

161   the system could contain antibiotic residues or select and/or enrich for ARGs (e.g., antibiotics, feed, manure

162   from poultry farms, drainage water, etc.). Management practices may involve the active use of antibiotics

163   or use of products containing antibiotic residues or ARGs (e.g., during the fertilisation stage of pond

164   preparation using poultry manure, the crop production using drainage water from aquaculture farms, etc.).

165

166   2.3. Plenary activity and follow up survey

167   The main findings from each group were summarised and presented back to all participants in a plenary

168   session, highlighting the key components of the system, major factors impeding production and good

169   management, drivers of ABU, the flow of antibiotics and residues in the system, and areas of disagreement

170   or inconsistency between the groups. Following this, a concluding exercise was conducted to compile

171   information on alternatives to antibiotics that had emerged during the mapping process in each group. In

172   addition, participants were asked to suggest any additional alternatives to antibiotics and interventions that

173   may reduce ABU by improving fish health and to rank these based on perceived feasibility and

174   effectiveness.

175   These discussions provided the foundation for a post-workshop survey to investigate the perceptions and

176   attitudes of the workshop participants, and additional stakeholders not in attendance, towards alternatives

177   to antibiotics and interventions aiming to improve fish health management practices and thus reduce ABU

178   (Supplementary file 1). The survey was structured and developed by the research team using information

179   gathered at the workshop, a review of existing literature, and expertise within the team. This resulted in a

180   list of 31 alternatives to antibiotics, or potential interventions, which were divided into eight groups. The

181   groups were: *farm management practices*; *new breeds and genetic improvement*; *disease diagnostics*;

182   *monitoring and control*; *biosecurity*; *rewards and incentives*; *education and training*; and *alternative*

183   *therapies and products*, e.g. vaccines, immunostimulants and others. Survey respondents were asked about

184   the perceived *feasibility* (short term, long term, or not possible to achieve), and *advantages and*

185   *disadvantages* (affordability, availability, ease and speed of application or implementation, and

186   effectiveness), and then to rank the interventions in terms of overall perceived importance to reduce ABU.

187   The survey was translated into Arabic and purposefully distributed by email to workshop participants via

188   SurveyMonkey, and in-person through the networks of the research team in the Kafrelsheikh Governorate

189   (completed either by email or face-to-face), aiming to reach aquaculture professionals, particularly farmers.

190   2.4. Analysis of maps, consensus map and validation

191   At the end of the workshop, each map developed by the three groups was photographed and translated by

192   the research team into a single, consensus digital diagram using the web-based Lucidchart software

193   (https://www.lucidchart.com/). The components of the consensus map were classified broadly into: (1)

194   *pre-harvest activities and production*; (2) *inputs*, such as feed and chemicals; (3) *waste products*; (4) *post-*

195   *harvest activities* and (5) *governance, roles and responsibilities of actors* taking decisions in the systems

196   particularly in regard to fish and public health.

3. Results

3.1 Mapping tilapia production systems

      3.1.1 Description of the system

Three workshop groups each constructed a map of the tilapia production system in the Nile Delta and it was apparent that each group had focused to varying degrees on distinct parts of the system, which probably reflected the different experiences of the participants in the groups. A consensus system map was constructed from these original hand-drawn maps (Figure 2), and this was relatively simple given the near complete absence of contradictory information. Tilapia is cultured almost exclusively in earthen grow-out ponds that are prepared initially by draining and removing sediment. The pond is then sun-dried and disinfected with lime, before filling with water. Thereafter, a bloom of microorganisms, most likely phytoplankton, zooplankton and bacteria, is initiated in the pond water through fertilisation with organic fertilisers such as poultry manure. This microbial community formed in the pond improves the water quality, provides additional nourishment to the fish, and may confer some protection against pathogens through niche closure and stimulation of the host immune system (Ahmad et al., 2017).

Grow-out earthen ponds (mean area ca. 1.3 ha) are usually located in the northern regions of the Nile Delta, with a main water supply of agricultural drainage water. Grow-out ponds are stocked either with mono-sex fry obtained directly from the local hatcheries, or by fingerlings that have been over-wintered in on-farm nursery ponds. The stocking of grow-out ponds usually starts in March. Almost all tilapia farmers cultivate tilapia in polycultures with two species of mullet, the flathead grey mullet (*Mugil cephalus*) and thin lip mullet (*Liza ramada*). Over the last 10 years, there has been a shift from mostly extensive production to semi-intensive and intensive systems due to the increased availability of hatcheries and commercial feed. Very few farmers have access to electricity and a limited number of farms use some type of aeration; most farmers use diesel pumps to pump water in and out of ponds. Feed mills provide feed formulated to contain a variety of raw ingredients, including soy bean meal (and oil extracted from the

222 beans), corn, and blood and fish meal. More than 90% of fish farms are using formulated commercial feed

223 and almost all farmers add feed to the pond manually. Some feeds are supplemented with additives, such

224 as probiotics, prebiotics, minerals, enzymes and antimycotoxins, which aim to prolong the shelf life,

225 increase digestibility and enhance the fish health status. These supplements may be added during

226 manufacture at the mill, but often are incorporated into the feed by farmers themselves. Supplements are

227 sourced from specialist retailers called 'aqua shops' marketing products exclusively for fish producers,

228 although these outlets may also sell products intended for use in other livestock.

229 The water entering the system is derived mainly from agricultural drainage from terrestrial crop production

230 (ca. 70% of farms), with a small proportion of farms deriving water from the Nile (ca. 20%) and

231 groundwater (ca. 10%). A small number of ponds have access to irrigation water, but this is illegal to use for

232 fish production, while some others have access to lake water. The water is channelled via a network of

233 interconnecting canals that link the fish farms. Few farms have access to groundwater to fill or top up

234 ponds during water exchange meaning most farms rely on the canals, but this water may contain

235 chemicals, sediment and microorganisms. All farms release wastewater into the canal network, which

236 exacerbates potential cross-contamination and means water of poor quality, containing potentially

237 pathogenic microorganisms and drug residues, may be introduced back into the tilapia ponds.

238 Fish exhibiting suspect clinical signs of infectious disease (e.g., bacterium or parasite) may be treated by

239 adding disinfectants to the pond water, such as formalin, copper sulphate or potassium permanganate.

240 During episodes of increased morbidity and mortality in the grow-out ponds, farmers often reduce the

241 water level in the pond before adding medications to the water. However, low diagnostic capacity,

242 stemming from a lack of trained veterinarians with expertise in aquatic animal health, and reliance on a

243 range of providers of varying quality for diagnostic support, means that diseases are likely to be commonly

244 misdiagnosed. In addition, antibiotics are available over the counter (i.e. without the need of a veterinary

245 prescription), from aqua shops and similar retailers, where they may be incorporated into feed, either by

246 the feed mill upon request or at the farms by the farmers. Opinions of whether feed mills add antibiotics to

247 feed or not were inconsistent across workshop groups. One group suggested this to be a common practice,

248   while another group maintained that feed mills do not add antibiotics to feed. However, further discussions

249   with a feed mill owner confirmed that feed mills did add medications to feeds upon request, but this was

250   rare and costly. Furthermore, antibiotics may be added to the culture water to treat diseased fish at

251   hatcheries and nurseries.

252   At harvest, fish are netted and transported to the markets without refrigeration or via a rudimentary cold

253   storage system (i.e. ice), which means the fish must be sold within a few hours (Figure 2, blue section). A

254   limited network of wholesalers supplies a range of sales outlets, including supermarkets, restaurants, fry

255   shops, and street side vendors.

256   The workshop maps showed that various government ministries are involved in governing the tilapia

257   production system. Ministerial involvement aims to promote and expand the industry. *The Ministry of*

258   *Agriculture and Land Reclamation* governs three main authorities: *The General Authority for Fish Resources*

259   *Development* (GAFRD), *The General Organization for Veterinary Services* (GOVS) and *The Agriculture*

260   *Research Centre* (ARC). The GAFRD drafts the legislation and regulations for fisheries and manages farm

261   licensing, aquaculture land use regulations, as well as extension and research services. The GOVS controls

262   the import/export of fish and conducts aquatic health surveillance, though this is not yet in place. The ARC

263   includes *The Animal Health Research Institute* (AHRI), which contains a department focused on the

264   management of fish diseases, and the *Central Laboratory for Aquaculture Research* (CLAR). *The Ministry of*

265   *Water Resources and Irrigation* controls water use and authorises the issuing of licences by *The Ministry of*

266   *Agriculture and Land Reclamation* to farmers to permit them to take water from the agricultural drainage

267   canals, though farmers rarely obtain such permits. It is illegal to take water from the Nile for aquaculture

268   use, although this may occur to a small degree.

269   *The Ministry of Environment* oversees the use of land for aquaculture purposes through the Egyptian

270   Environment Affairs Agency (EEAA). The establishment of new fish ponds must be subject to an

271   Environmental Impact Assessment (EIA), which is reviewed by the EEAA. The scope and depth of the EIA is

272   determined by the EEAA on the basis of the information provided in the application, and will vary

273   depending on factors such as whether the farm is situated in an urban setting, or an ecologically sensitive

274   or protected area.

275   *The National Food Safety Authority* (NFSA) carries out inspections and performs laboratory analysis of fish

276   samples for the domestic market and to meet export conditions as required. *The Ministry of Higher*

277   *Education and Scientific Research* includes educational and research institutes that support the tilapia

278   sector with qualified aquaculture specialists and research outputs to improve the industry.

279

280   Boundaries to the tilapia production system map include: the sources of ingredients for feed, including feed

281   additives; international and overseas agencies of governance; crop production; poultry (and other

282   livestock) production; production of chemical inputs (e.g., disinfectants, antibiotics); feed supplements; ice,

283   energy, and brood stock; domestic wastes that may contaminate water sources; production of equipment

284   such as nets and tanks; sales and marketing outlets; and service providers (e.g. veterinarians etc.).

285

286   <u>3.1.2 Factors that impact production and fish health</u>

287   Different stakeholders participating in the workshop emphasised factors that impact production and fish

288   health. Producers mentioned several factors that often appeared to be interconnected, including the high

289   prices of the inputs and dependency on value chain stakeholders, fluctuation of market prices of the

290   products, access to clean water and irrigation systems, losses due to diseases, and regulations such as the

291   restrictions in the irrigation systems and the marketisation of products.

292   Fish health and management stakeholders mentioned occasional moderate mortality rates and outbreaks,

293   but disease was often described to be linked to management deficiencies. In particular, poor water quality

294   was described to be a key factor impacting fish health by different stakeholders. Further, workshop

295   participants observed that pressure to produce a timely harvest favoured short-term solutions and

296   prophylactic use of veterinary products, instead of investing or focusing on improved disease prevention

297   and control, management and biosecurity.

298   Stakeholders mentioned the generalised lack of monitoring for water quality parameters at the farm level,

299   and many producers rely solely on organoleptic characteristics to determine water quality. Similarly, there

300   is no widespread use of disease diagnostic tests and antibiotic susceptibility testing, which often results in

301   recurrence of infectious diseases and ineffectual empirical treatments.

302

303   3.1.3 Antibiotics in the system

304   Fish mortality was the main driver directly leading to ABU, generally described to be applied to treat stocks

305   with evidence of clinical signs, but participants also mentioned occasional prophylaxis when neighbouring

306   farmers were experiencing an outbreak suspected to be due to an infectious disease. The most common

307   product referenced was oxytetracycline (a tetracycline), though sulphonamides and other antibiotic

308   substances were also mentioned in discussions. During a visit to two aqua shops in the locality of the

309   workshop by the research team, products containing dihydrostreptomycin (an aminoglycoside),

310   oxytetracycline, ciprofloxacin (a fluoroquinolone), erythromycin (a macrolide), and sulphadimidine (a

311   sulphonamide) were observed for sale.

312   One direct way antibiotics enter the system is from the aqua shops and similar retailers. Antibiotics are

313   used to treat diseases and these may be added to feed or to the pond water particularly where water-body

314   volumes are smaller such as at hatcheries and nurseries, though grow-out pond water levels can be

315   reduced for treatment in this way by releasing water into the agricultural drainage canals. Antibiotics may

316   also be introduced into the tilapia production system as residues in wastewater derived from other

317   systems, including domestic waste, which enters into the agricultural drainage canals used subsequently by

318   fish farmers to replenish the ponds. Thus, these canals represent a potential source and reservoir of

319   antibiotic residues, which allows them to be transported widely across the system. A further possible

320   source of antibiotic residues and resistant bacteria entering the tilapia production system may be the use of

321   organic wastes, such as untreated poultry manure, to fertilise the ponds prior to stocking.

322

323 Having plotted the movements of antibiotics within the system, the workshop groups proceeded to identify

324 hotspots where the presence of antibiotics or residues, or other conditions or compounds, may select for

325 resistant organisms containing ARGs. Hotspots identified included: *hatcheries and nurseries*, where

326 antibiotics may be applied to prevent or treat disease events; *the drainage canals* and the water contained

327 within, where residues from various systems are transported and may accumulate; and the *grow-out ponds*

328 at the farms, where antibiotics may be introduced during fertilisation with poultry manure, during

329 application of medicated feeds, from the introduction of contaminated canal water, or where the

330 application of co-selectors of ARGs such as disinfectants are applied. Further hotspots included the *feed*

331 *mills*, where antibiotics may be incorporated into fish feed, and in *crop and poultry production*, where

332 antibiotics may be applied to prevent or mitigate infectious diseases or as growth promotors.

333

334 <u>3.2. Attitudes and perceptions of survey respondents</u>

335 A total of 69 responses to the survey were received within six weeks of the workshop. Respondents

336 represented a variety of stakeholders as described in Figure 3, but primarily fish farmers (n=51). Of the 69

337 respondents to the survey, 74% were not present at the workshop. Response rate for individual questions

338 ranged from 84% to 100%, with 84% of respondents completing the survey in full. All 51 farmers were

339 surveyed face-to-face and completed the survey in full. A summary of the responses from the entire survey

340 is presented in Figures 4a and 4b.

341

342 Improving water exchange practices and storing feed in more suitable conditions were perceived to be

343 most achievable in the short-term to improve fish health, mainly because these actions were felt to be

344 effective and relatively easy and inexpensive to implement. Using pond fertilisers most appropriately was

345 perceived to be a possible solution to be implemented in the short-term, but only 10% of respondents

346 thought that this approach would actually be effective in improving fish production and reducing ABU.

347 There was an agreement that pond modifications allowing fish to select thermal preference (Cerqueira et

348 al., 2016; Tabin et al., 2018) could be achieved and such changes were felt strongly to be effective, but

349   there was a split in opinion on the possible timescale required for this intervention, which was also

350   perceived to be expensive. More than 40% did not know whether better quality, nutritious feed than what

351   is currently available could be achieved (e.g., by sourcing higher quality ingredients and improved

352   manufacturing practices) and this likely reflects the lack of knowledge among respondents regarding the

353   potential impact on productivity and fish health, or what is needed for this to be accomplished.

354   Furthermore, it was clear that alternative sources to the agricultural drainage water were not thought to be

355   possible due to the current restrictions on water use imposed by existing legislation, despite being

356   identified to be an effective way to improve fish production and reduce ABU.

357

358   There was strong support for the development of genetically improved fish for disease resistance as a long-

359   term goal and an effective strategy to reduce disease and ABU, but disease-resistant strains were

360   recognised to be unavailable and costly to develop. There was strong support for the introduction of rapid

361   diagnostics and a role for the routine application of antibiotic sensitivity testing as effective ways to reduce

362   disease and ABU, although there was a split in opinion on whether this was achievable. The majority of

363   respondents indicated that such tests were unavailable, with others saying the prohibiting factor in their

364   implementation was high cost.

365

366   Respondents perceived short-term benefits to be delivered by monitoring water quality parameters, such

367   as dissolved oxygen levels and water 'transparency', and making an inventory of fish clinical signs and

368   behaviour to inform diagnosis, which largely stemmed from being relatively easy to implement, although

369   there were concerns around the cost of tests. Improved biosecurity was perceived to be achievable by most

370   respondents as a way to reduce disease outbreaks and ABU, with most thinking this would be effective.

371   Although almost a third of respondents felt this would not be possible due to the cost, and the difficulty

372   and time needed to achieve improvements.

373

374   There was support for developing export markets, certification and quality assurance schemes as long-

375   term, effective strategies to reduce disease outbreaks and ABU. However, many respondents were unsure

376   of the benefits and barriers to developing export markets. Producer associations and cooperatives were

377   thought by many respondents to be easy to establish, though many respondents were unsure whether this

378   could be realised. The questions pertaining to education and training showed an appetite amongst

379   respondents for learning and acquiring new skills, and this was seen as achievable in the short term, easy

380   and effective for reducing ABU and disease outbreaks, although concerns were raised about cost (mostly)

381   and a lack of availability.

382

383   Various alternatives to antibiotics for preventing and treating disease had been proposed during the

384   workshop discussions. Amongst these, the use of vitamin C, probiotics and immunostimulants all received

385   strong support and were perceived to be capable of delivering benefits in the short-term due to their

386   effectiveness, low cost, ease of availability and ease of use. Application of natural plant extracts was

387   perceived to offer benefits for similar reasons; however, their use was envisaged further in the future. Few

388   respondents viewed vaccination positively in its potential effectiveness to reduce disease and ABU, possibly

389   due to lack of availability, and there was little knowledge of the potential of bacteriophage therapy in

390   disease control.

391

392   Finally, in a ranking exercise, improved farm management practices and biosecurity were ranked highest in

393   terms of importance to reduce ABU and improve aquaculture, with (presumably overt) rewards and

394   incentives perceived to be least important by a considerable margin (Figure 5).

395

4. Discussion

The aim of this study was to identify potential interventions that may reduce ABU in farmed tilapia production in Egypt using a participatory systems-thinking approach with key stakeholders from this industry. A consensus map of the farmed tilapia production system was created and used to identify possible hotspots of ABU and emergence of ABR, routes of antibiotic flow through the system, and interactions with other systems. Thereafter, approaches that would promote fish health and reduce or prevent ABU were proposed, discussed and their perceived feasibility assessed by key stakeholders through a survey that followed the workshop.

Previous assessments of the tilapia sector in Egypt using value-chain analysis have provided information concerning structure and performance, and identified key constraints within the sector (Macfadyen et al., 2012; Eltholth et al., 2015). Here, a systems-thinking approach was taken to integrate the multi-level aspects of the system, such as governance and the environment, and to provide a framework for exploring how a proposed intervention to mitigate ABR at one point in the system might affect the functioning of the entire system. Such an approach is needed due to the complexity of the system, and the participatory nature of the approach is beneficial because it can capture a range of views, leads to a sense of collective responsibility towards a problem, and provides a feeling of shared ownership towards solutions (Evans and Terrey, 2016; Blomkamp, 2018). Mapping the system provided the means to visualise ABU in the sector, which allowed for identification of how and where in the system antibiotics are introduced, how and where they are transmitted, and the points in the system that represent potential hotspots for the emergence and selection of ABR.

In a previous study, a similar approach was used to identify hotspots for the emergence of ABR in striped catfish and shrimp farming in Vietnam (Brunton et al., 2019). Similarities between the aquaculture production systems in Egypt and Vietnam emerged with respect to the locations of hotspots for the emergence of ABR, with culture ponds, feed mills and markets identified in both exercises (Brunton et al., 2019). Moreover, these hotspots hold the potential for human exposure to antibiotic residues, ARGs and

421   antibiotic-resistant bacteria. Other studies have reported similar findings, with a variety of antibiotic

422   residues being detected in aquaculture ponds in Vietnam (Le et al., 2005; Andrieu et al., 2015; Giang et al.,

423   2015; Nakayama et al., 2017), and antibiotic-resistant bacteria detected in aquaculture ponds in Egypt

424   (Ishida et al., 2010; El-Gohary et al., 2020).

425

426   Mapping the farmed tilapia production system highlighted the interaction between aquaculture and

427   agricultural systems, with water contaminated with antibiotic residues able to permeate the whole delta

428   and move between other systems, including crop and terrestrial animal production systems. For example,

429   poultry manure and litter are commonly used as fertiliser for fish farming due to its nutritional value

430   (Mahmoud and Abdel-Mohsein, 2019), but it is rarely treated (e.g. pasteurised) before use (Eltholth et al.,

431   2015). This can represent a possible source of antibiotic residues (Petersen et al., 2002), in particular

432   tetracyclines, which are used extensively in poultry production in Egypt (Mahmoud and Abdel-Mohsein,

433   2019). Tetracycline concentrations exceeding European Union maximum residue limits (100 µg/kg) have

434   been detected in wild and farmed tilapia in the Nile delta region, with greater concentrations in farmed

435   versus wild samples suggested to be due to the use of contaminated poultry manure as pond fertiliser

436   (Mahmoud and Abdel-Mohsein, 2019). Indeed, high concentrations of tetracycline residues were detected

437   even on fish farms with no recent history of using this antibiotic (Mahmoud and Abdel-Mohsein, 2019),

438   indicating soil and groundwater may act as reservoirs for antibiotic residues (Boxall et al., 2003). In

439   addition, high levels of ARGs have been detected in poultry manure and litter, and these genes may be

440   carried by pathogens (Abu-Elala et al., 2015; Mahmoud and Abdel-Mohsein, 2019) and commensal bacteria

441   alike.

442

443   Availability of and accessibility to irrigation canal water was a key challenge that emerged during both the

444   workshop discussions and from the survey data. Agricultural drainage water is the main water supply for

445   most fish farms in this region of Egypt, but it represents a major biosecurity risk and is a key potential

446   source of antibiotic residues, ARGs and antibiotic-resistant bacteria that may contaminate the culture

447   ponds (Eltholth et al., 2015). The use of irrigation water in fish ponds is not allowed because it is prioritised

448   for agricultural use (GAFRD Law No 124/1983). Since agricultural drainage water can be detrimental to fish

449   health and growth, it has been suggested instead to use irrigation water primarily in aquaculture and then

450   use the aquaculture effluent in agriculture as a source of water and organic fertiliser (Henriksson et al.,

451   2017). While this would not stop chemical and biological hazards from fish ponds being disseminated to the

452   wider environment, it might lead to an overall reduction in the transmission of such hazards, if water

453   quality improvements, including lower levels of pathogens, lead to reduced application of antibiotics in

454   aquaculture. This is worthy of consideration but may not be possible in Egypt, as the land set aside for

455   aquaculture is mostly downstream of agricultural crops. According to workshop participants, about 20 to

456   30% of water is exchanged every day under normal circumstances; however, the frequency and volume of

457   water exchanged depend on the stage of production, size of fish and the season (Eltholth et al., 2015). The

458   main purpose of pumping water is to increase the dissolved oxygen level in pond water. During outbreaks,

459   some farmers reduce the level of pond water to the minimum to administer a treatment to the fish

460   (Eltholth et al., 2015). Many respondents to the survey reported that more frequent water exchanges

461   would be feasible in the short term, and felt that this would be effective in improving fish health. This may

462   not be the case where the quality of incoming water is inadequate, and thus may be counter-productive,

463   which could indicate some misunderstanding among respondents of the water quality problems and how

464   best to solve them.

465

466   Demand for fish is increasing in Egypt due to a growing population and increased per capita consumption in

467   the last two decades, likely due to economically incentivised changes in consumer preferences and

468   increased accessibility (i.e., low-cost domestic fish production, improvements in distribution networks, etc.)

469   (Murphy et al., 2020). Indirect price controls on tilapia have been imposed by the government through the

470   introduction of an export tax for tilapia, and this policy has created tension between a government aiming

471   to ensure a reliable, low cost and secure supply of animal protein for the population in the country and

472   tilapia producers keen to maximise profits. Survey respondents expressed a strong desire to explore and

473   expand exporting opportunities, and it was suggested that this could act as a driver to reduce ABU by

474   raising production standards and allowing farmers to sell fish at higher prices, as observed elsewhere

475   (Nguyen and Jolly, 2020). Currently, a notable barrier to exporting tilapia from Egypt is the need for a

476   comprehensive residue monitoring system and disease testing framework, both of which are required for

477   export to the European Union and the United States (Fitzsimmons, 2008; Eltholth et al., 2015). In addition,

478   the costs associated with higher standards of production, limited processing facilities and ability to add

479   value, and lack of by-product industries represent important barriers to export (Fitzsimmons, 2008; Eltholth

480   et al., 2015). Previous research has shown the importance of the local market, where tilapia consumption is

481   greater amongst the communities in high production areas than in non-production areas where fish may be

482   available only once per week on market day (Eltholth et al., 2015). Currently the tilapia market is

483   dominated by a few large wholesalers who control the price (Soliman and Yacout, 2016), and the national

484   government seeks to ensure the tilapia industry offers food security for the population, meaning

485   production for the domestic market is prioritised. Indeed, the building of government aquaculture farms,

486   such as the Birkat Ghalioun fish pond project (Feidi, 2018), illustrates the government's plans to meet the

487   increased demand for fish for the population (El-Gohary et al., 2020). However, it is conceivable that

488   greater revenues resulting from exporting tilapia could provide the income necessary to secure the supply

489   of alternative high-protein foodstuffs.

490

491   Improvements to diagnostic capacity and a need for alternatives to antibiotics were frequently mentioned

492   at the workshop and these deficiencies are often highlighted by aquaculture farmers in LMICs (Ninawe et

493   al., 2017; Stentiford et al., 2017; Garza et al., 2019). While improved diagnostics were considered

494   favourably by respondents, the consensus was that cost was a barrier and it is difficult to envisage how this

495   situation will improve to meet the needs of farmers in the near future, given the significant investment that

496 would be necessary. The use of probiotics and immunostimulants may offer a feasible and relatively

497 inexpensive option to reduce the need for antibiotics by improving the health and welfare of stocks

498 (Chauhan and Singh, 2019). There have been numerous trials with these products in tilapia, for example

499 dietary supplementation of Nile tilapia with *Bacillus subtilis* and *Saccharomyces cerevisiae* was associated

500 with improvements in selected measures of immunity and an enhancement of fish flesh quality (Elsabagh

501 et al., 2018; Opiyo et al., 2019). In addition, probiotics can be used to modify the microbial composition of

502 pond water to improve water quality (De Schryver et al., 2014). Still, much more needs to be done to

503 ensure probiotics are tested appropriately and confirmed to be effective under farm conditions (Partridge,

504 2016; Knipe et al., 2020).

505

506 Meanwhile, few survey respondents viewed vaccination positively for its potential effectiveness to reduce

507 disease and ABU, even though vaccination programmes can lead to massive reductions in ABU, such as has

508 been seen in Atlantic salmon farming around Scotland and Norway (Sommerset et al., 2005; Adams, 2019).

509 It was discussed at the workshop that there are few vaccines available for tilapia, and that these are

510 currently injection vaccines, whereas immersion vaccination would be more appropriate. Although there

511 was little knowledge of the potential of bacteriophage in disease control, this is less surprising given it is not

512 an established technology like vaccination (Gon Choudhury et al., 2017). The lack of appreciation for the

513 potential of vaccination programmes to improve fish health may be due, in part, to a lack of awareness and

514 lack of availability of vaccines for tilapia diseases. However, it is encouraging that there was an appreciation

515 by survey respondents for the importance of education and training to improve the ABR situation in tilapia

516 farming, indicating a possible avenue to raising attention to the success of vaccination programmes in

517 improving fish health and reducing ABU. More broadly, this appetite for learning provides a platform that

518 can be used to improve many aspects of tilapia production in the country. Indeed, the impact of training

519 farmers in best management practices (BMP) has been assessed in the aquaculture sector in Egypt (Dickson

520 et al., 2016; Henriksson et al., 2017). Providing farmers with BMP training improves farm profitability

521 though not necessarily productivity, and the considerable economic, social and environmental gains from

522 investment in training of fish farmers in Egypt is well recognised (Dickson et al., 2016). Indeed, Henriksson

523 et al. (2017) used lifecycle assessment to show that BMP training of tilapia farmers in Egypt reduced

524 lifecycle environmental impacts by 22%.

525

526 A limitation of this present study was the absence of policy makers from government agencies, as this

527 would have provided broader context and a better understanding of the decision-making processes at

528 national level. However, the presence of government representatives could have altered the dynamic of

529 the workshop, and influenced the responses of the ground-level stakeholders identified for inclusion in this

530 present study. It was important to capture the views of these stakeholders on the acceptability and

531 feasibility of the interventions, given that the proposed interventions will have greatest impact upon them

532 and will most likely need to be implemented by them. Having captured these views, engagement with

533 government is a priority and is essential to understand how best to influence policy, particularly on water

534 use and the constraints to exporting tilapia to different territories, and how any barriers may be

535 surmounted. In addition, this engagement would be useful for identifying potential resources and means to

536 deliver the desired increase in diagnostic capacity. Therefore, a smaller, focused follow-up workshop is

537 proposed to include a broader range of stakeholders, including those representing relevant government

538 agencies and other systems like poultry production, while also ensuring the key opinions from attendees at

539 our initial workshop are represented through inclusion of a subset of delegates from the fish farming

540 stakeholder community. It is by this approach that acceptable, sustainable interventions to reduce ABU,

541 and thus the problem of bacterial ABR, can be introduced to deliver beneficial change in the aquaculture

542 sector.

543

544 A further limitation of this study is that participants and respondents lack of knowledge or experience with

545 specific interventions may have influenced their responses to the survey. Though some information was

546   provided in the descriptions of each intervention in the questionnaire, this may still have been insufficient

547   for responders to make an informed judgement. This could potentially skew responses both ways,

548   depending on how optimistic or pessimistic the responder may be towards a new intervention. This could

549   be mitigated in future studies by providing more opportunities for training and discussion to achieve a

550   shared understanding of the interventions prior to collecting participants' views. Language may represent a

551   further barrier to understanding and, though reasonable steps were taken to mitigate this risk, it is not

552   inconceivable that misunderstanding could have happened. The workshop was led in English by the

553   research team, with concurrent translation into Arabic by a member of the research team fluent in both

554   languages. Each workshop group was facilitated in English with a translator present. The survey was written

555   in English and translated into Arabic before dissemination, whilst responses provided in Arabic were

556   translated back into English by a member of our research team.

557

558   The range of suggested interventions above can be enacted by various stakeholders in the tilapia

559   production system in Egypt. The benefit of the systems-thinking approach is that it allows for consequences

560   of actions to be assessed and predicted, while also providing a means to select points in the system where

561   the effectiveness of interventions could be evaluated through the collection of empirical data, thus helping

562   to focus limited resources most effectively (Adam and de Savigny, 2012; Peters, 2014). These data can be

563   used to confirm or quantify risks and could consist of measures such as concentration levels of antibiotic

564   residues or presence of ARGs in the bacterial populations in the system.

565

566   In conclusion, this present study provides a foundation for the perceived acceptability and feasibility of

567   possible interventions that can reduce ABU in tilapia production in Egypt and other countries with similar

568   production systems and thus mitigate against the broader problems posed by ABR. This will help the sector

569   to remain productive and increase its resilience to ABR but also to infectious diseases, while securing

570   livelihoods and ensuring farmed tilapia is a safe and nutritious source of food. Systems-thinking and

571   participatory approaches to create systems maps are important when determining the points within

572   systems where interventions can be implemented, with feasibility and stakeholder acceptance being crucial

573   considerations for the success of any intervention (Siokou et al., 2014). These same system maps can be

574   used to assist in selecting points to monitor and assess the effectiveness of proposed interventions, a

575   better understanding of which is crucial if we are to be successful in reducing overall ABU and tackling the

576   global problem of ABR (Wernli et al., 2020).

577

583

584   **Ethical approval**

585   Ethics approval for this work was granted by the Social Science Research Ethical Review Board at the Royal

586   Veterinary College, under reference number: URN SR2020-0249.

587

593   **References**

594   Abu-Elala, N., Abdelsalam, M., Marouf, S., Setta, A., 2015. Comparative analysis of virulence genes,
595   antibiotic resistance and gyrB-based phylogeny of motile Aeromonas species isolates from Nile
596   tilapia and domestic fowl. Lett Appl Microbiol 61, 429-436.
597   Adam, T., de Savigny, D., 2012. Systems thinking for strengthening health systems in LMICs: need for a
598   paradigm shift. Health Policy and Planning 27, iv1-iv3.
599   Adams, A., 2019. Progress, challenges and opportunities in fish vaccine development. Fish Shellfish Immun
600   90, 210-214.
601   Ahmad, I., Babitha Rani, A.M., Verma, A.K., Maqsood, M., 2017. Biofloc technology: an emerging avenue in
602   aquatic animal healthcare and nutrition. Aquaculture International 25, 1215-1226.
603   Ali, S.E., Jansen, M.D., Mohan, C.V., Delamare-Deboutteville, J., Charo-Karisa, H., 2020. Key risk factors,
604   farming practices and economic losses associated with tilapia mortality in Egypt. Aquaculture 527,
605   735438.
606   Aly, S.M., 2013. A review of fish diseases in the Egyptian aquaculture sector. Working report. WorldFish,
607   Penang, Malaysia,.
608   Andrieu, M., Rico, A., Phu, T.M., Huong, D.T.T., Phuong, N.T., Van den Brink, P.J., 2015. Ecological risk
609   assessment of the antibiotic enrofloxacin applied to Pangasius catfish farms in the Mekong Delta,
610   Vietnam. Chemosphere 119, 407-414.
611   Blomkamp, E., 2018. The promise of co-design for public policy. Australian Journal of Public Administration
612   77, 729-743.
613   Brunton, L.A., Desbois, A.P., Garza, M., Wieland, B., Mohan, C.V., Häsler, B., Tam, C.C., Le, P.N.T., Phuong,
614   N.T., Van, P.T., Nguyen-Viet, H., Eltholth, M.M., Pham, D.K., Duc, P.P., Linh, N.T., Rich, K.M., Mateus,
615   A.L.P., Hoque, M.A., Ahad, A., Khan, M.N.A., Adams, A., Guitian, J., 2019. Identifying hotspots for
616   antibiotic resistance emergence and selection, and elucidating pathways to human exposure:
617   Application of a systems-thinking approach to aquaculture systems. Science of The Total
618   Environment 687, 1344-1356.
619   Cabello, F.C., Godfrey, H.P., Buschmann, A.H., Dölz, H.J., 2016. Aquaculture as yet another environmental
620   gateway to the development and globalisation of antimicrobial resistance. Lancet Infect Dis 16,
621   e127-e133.
622   Cantas, L., Shah, S., Cavaco, L., Manaia, C., Walsh, F., Popowska, M., Garelick, H., Bürgmann, H., Sørum, H.,
623   2013. A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the
624   global environmental microbiota. Frontiers in Microbiology 4.
625   Cerqueira, M., Rey, S., Silva, T., Featherstone, Z., Crumlish, M., MacKenzie, S., 2016. Thermal preference
626   predicts animal personality in Nile tilapia Oreochromis niloticus. Journal of Animal Ecology 85,
627   1389-1400.
628   Chauhan, A., Singh, R., 2019. Probiotics in aquaculture: a promising emerging alternative approach.
629   Symbiosis 77, 99-113.
630   Chuah, L.-O., Effarizah, M.E., Goni, A.M., Rusul, G., 2016. Antibiotic Application and Emergence of Multiple
631   Antibiotic Resistance (MAR) in Global Catfish Aquaculture. Current Environmental Health Reports 3,
632   118-127.
633   De Schryver, P., Defoirdt, T., Sorgeloos, P., 2014. Early Mortality Syndrome Outbreaks: A Microbial
634   Management Issue in Shrimp Farming? PLOS Pathogens 10, e1003919.
635   Dickson, M., Nasr-Allah, A., Kenawy, D., Kruijssen, F., 2016. Increasing fish farm profitability through
636   aquaculture best management practice training in Egypt. Aquaculture 465, 172-178.
637   Done, H.Y., Venkatesan, A.K., Halden, R.U., 2015. Does the Recent Growth of Aquaculture Create Antibiotic
638   Resistance Threats Different from those Associated with Land Animal Production in Agriculture?
639   AAPS J 17, 513-524.
640   El-Gohary, F.A., Zahran, E., Abd El-Gawad, E.A., El-Gohary, A.H., M. Abdelhamid, F., El-Mleeh, A.,
641   Elmahallawy, E.K., Elsayed, M.M., 2020. Investigation of the Prevalence, Virulence Genes, and

642   Antibiogram of Motile Aeromonads Isolated from Nile Tilapia Fish Farms in Egypt and Assessment
643   of their Water Quality. Animals 10, 1432.

644 El-Sayed, A.-F.M., 2017. Tilapia Co-culture in Egypt. In: Perschbacher, P.W., Stickney, R.R. (Eds.), Tilapia in
645   Intensive Co-culture. 211-236. https://doi.org/10.1002/9781118970652.ch13

646 Elsabagh, M., Mohamed, R., Moustafa, E.M., Hamza, A., Farrag, F., Decamp, O., Dawood, M.A.O., Eltholth,
647   M., 2018. Assessing the impact of Bacillus strains mixture probiotic on water quality, growth
648   performance, blood profile and intestinal morphology of Nile tilapia, Oreochromis niloticus.
649   Aquaculture Nutrition 24, 1613-1622.

650 Eltholth, M., Fornace, K., Grace, D., Rushton, J., Häsler, B., 2015. Characterisation of production, marketing
651   and consumption patterns of farmed tilapia in the Nile Delta of Egypt. Food Policy 51, 131-143.

652 Evans, M., Terrey, N., 2016. Co-design with citizens and stakeholders. Evidence-based Policymaking in the
653   Social Sciences: Methods that Matter, 243-262.

654 FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action., Rome.

655 FAO, 2021a, FAO Commodities dataset 1976-2018, Fisheries and Aquaculture Information and Statistics
656   Branch. (online)
657   http://www.fao.org/figis/servlet/TabLandArea?tb_ds=Commodities&tb_mode=TABLE&tb_act=SEL
658   ECT&tb_grp=COUNTRY (Accessed) 02/02/21

659 FAO, 2021b, FAO Global Aquaculture Production dataset 1950-2018, Fisheries and Aquaculture Information
660   and Statistics Branch. (online) http://www.fao.org/fishery/statistics/global-aquaculture-
661   production/query/en (Accessed) 02/02/21

662 Feidi, I., 2018. Will the New Large-Scale Aquaculture Projects Make Egypt Self Sufficient In Fish Supplies? .
663   Mediterranean Fisheries and Aquaculture Research 1, 31-41.

664 Fitzsimmons, K., 2008. Tilapia Product Quality and New Product Forms for International Markets. 8th
665   International Symposium on Tilapia in Aquaculture.,
666   https://www.researchgate.net/profile/Kevin_Fitzsimmons/publication/228832408_TILAPIA_PROD
667   UCT_QUALITY_AND_NEW_PRODUCT_FORMS_FOR_INTERNATIONAL_MARKETS/links/00b7d53528f
668   3fcdf79000000.pdf.

669 Garza, M., Mohan, C.V., Rahman, M., Wieland, B., Häsler, B., 2019. The role of infectious disease impact in
670   informing decision-making for animal health management in aquaculture systems in Bangladesh.
671   Prev Vet Med 167, 202-213.

672 Giang, C.N.D., Sebesvari, Z., Renaud, F., Rosendahl, I., Hoang Minh, Q., Amelung, W., 2015. Occurrence and
673   Dissipation of the Antibiotics Sulfamethoxazole, Sulfadiazine, Trimethoprim, and Enrofloxacin in the
674   Mekong Delta, Vietnam. PLoS One 10, e0131855.

675 Gon Choudhury, T., Tharabenahalli Nagaraju, V., Gita, S., Paria, A., Parhi, J., 2017. Advances in
676   Bacteriophage Research for Bacterial Disease Control in Aquaculture. Reviews in Fisheries Science
677   & Aquaculture 25, 113-125.

678 Henriksson, P.J.G., Dickson, M., Allah, A.N., Al-Kenawy, D., Phillips, M., 2017. Benchmarking the
679   environmental performance of best management practice and genetic improvements in Egyptian
680   aquaculture using life cycle assessment. Aquaculture 468, 53-59.

681 Henriksson, P.J.G., Rico, A., Troell, M., Klinger, D.H., Buschmann, A.H., Saksida, S., Chadag, M.V., Zhang, W.,
682   2018. Unpacking factors influencing antimicrobial use in global aquaculture and their implication
683   for management: a review from a systems perspective. Sustainability science 13, 1105-1120.

684 Ishida, Y., Ahmed, A.M., Mahfouz, N.B., Kimura, T., El-Khodery, S.A., Moawad, A.A., Shimamoto, T., 2010.
685   Molecular Analysis of Antimicrobial Resistance in Gram-Negative Bacteria Isolated from Fish Farms
686   in Egypt. Journal of Veterinary Medical Science 72, 727-734.

687 Knipe, H., Temperton, B., Lange, A., Bass, D., Tyler, C.R., 2020. Probiotics and competitive exclusion of
688   pathogens in shrimp aquaculture. Reviews in Aquaculture n/a.

689 Le, T.X., Munekage, Y., Kato, S.-i., 2005. Antibiotic resistance in bacteria from shrimp farming in mangrove
690   areas. Science of The Total Environment 349, 95-105.

691 Lulijwa, R., Rupia, E.J., Alfaro, A.C., 2020. Antibiotic use in aquaculture, policies and regulation, health and
692   environmental risks: a review of the top 15 major producers. Reviews in Aquaculture 12, 640-663.

693 Macfadyen, G., Nasr-Alla, A.M., Al-Kenawy, D., Fathi, M., Hebicha, H., Diab, A.M., Hussein, S.M., Abou-Zeid,
694   R.M., El-Naggar, G., 2012. Value-chain analysis — An assessment methodology to estimate Egyptian
695   aquaculture sector performance. Aquaculture 362-363, 18-27.
696 Mahmoud, M.A.M., Abdel-Mohsein, H.S., 2019. Hysterical tetracycline in intensive poultry farms
697   accountable for substantial gene resistance, health and ecological risk in Egypt- manure and fish.
698   Environmental Pollution 255, 113039.
699 Murphy, S., Charo-Karisa, H., Rajaratnam, S., Cole, S.M., McDougall, C., Nasr-Allah, A.M., Kenawy, D., Abou
700   Zead, M.Y., van Brakel, M.L., Banks, L.K., Ibrahim, N., 2020. Selective breeding trait preferences for
701   farmed tilapia among low-income women and men consumers in Egypt: Implications for pro-poor
702   and gender-responsive fish breeding programmes. Aquaculture 525, 735042.
703 Nakayama, T., Tuyet Hoa, T.T., Harada, K., Warisaya, M., Asayama, M., Hinenoya, A., Lee, J.W., Phu, T.M.,
704   Ueda, S., Sumimura, Y., Hirata, K., Phuong, N.T., Yamamoto, Y., 2017. Water metagenomic analysis
705   reveals low bacterial diversity and the presence of antimicrobial residues and resistance genes in a
706   river containing wastewater from backyard aquacultures in the Mekong Delta, Vietnam.
707   Environmental pollution (Barking, Essex : 1987) 222, 294-306.
708 Nguyen, T.A.T., Jolly, C.M., 2020. Global value chain and food safety and quality standards of Vietnam
709   pangasius exports. Aquaculture Reports 16, 100256.
710 Ninawe, A.S., Hameed, A.S.S., Selvin, J., 2017. Advancements in diagnosis and control measures of viral
711   pathogens in aquaculture: an Indian perspective. Aquaculture International 25, 251-264.
712 Opiyo, M.A., Jumbe, J., Ngugi, C.C., Charo-Karisa, H., 2019. Dietary administration of probiotics modulates
713   non-specific immunity and gut microbiota of Nile tilapia (Oreochromis niloticus) cultured in low
714   input ponds. International Journal of Veterinary Science and Medicine 7, 1-9.
715 Osman, K.M., Al-Maary, K.S., Mubarak, A.S., Dawoud, T.M., Moussa, I.M.I., Ibrahim, M.D.S., Hessain, A.M.,
716   Orabi, A., Fawzy, N.M., 2017. Characterization and susceptibility of streptococci and enterococci
717   isolated from Nile tilapia (Oreochromis niloticus) showing septicaemia in aquaculture and wild sites
718   in Egypt. BMC Veterinary Research 13, 357.
719 Osman, K.M., Ali, M.N., Radwan, I., ElHofy, F., Abed, A.H., Orabi, A., Fawzy, N.M., 2016. Dispersion of the
720   Vancomycin Resistance Genes vanA and vanC of Enterococcus Isolated from Nile Tilapia on Retail
721   Sale: A Public Health Hazard. Frontiers in Microbiology 7.
722 Partridge, G., 2016. Testing the efficacy of probiotics for disease control in aquaculture. Microbiology
723   Australia 37, 122-123.
724 Peters, D.H., 2014. The application of systems thinking in health: why use systems thinking? Health
725   Research Policy and Systems 12, 51.
726 Petersen, A., Andersen, J.S., Kaewmak, T., Somsiri, T., Dalsgaard, A., 2002. Impact of integrated fish farming
727   on antimicrobial resistance in a pond environment. Applied and environmental microbiology 68,
728   6036-6042.
729 Rico, A., Phu, T.M., Satapornvanit, K., Min, J., Shahabuddin, A.M., Henriksson, P.J.G., Murray, F.J., Little,
730   D.C., Dalsgaard, A., Van den Brink, P.J., 2013. Use of veterinary medicines, feed additives and
731   probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture
732   412-413, 231-243.
733 Robinson, T.P., Bu, D.P., Carrique-Mas, J., Fèvre, E.M., Gilbert, M., Grace, D., Hay, S.I., Jiwakanon, J., Kakkar,
734   M., Kariuki, S., Laxminarayan, R., Lubroth, J., Magnusson, U., Thi Ngoc, P., Van Boeckel, T.P.,
735   Woolhouse, M.E.J., 2016. Antibiotic resistance is the quintessential One Health issue. Transactions
736   of The Royal Society of Tropical Medicine and Hygiene 110, 377-380.
737 Rüegg, S.R., Nielsen, L.R., Buttigieg, S.C., Santa, M., Aragrande, M., Canali, M., Ehlinger, T., Chantziaras, I.,
738   Boriani, E., Radeski, M., Bruce, M., Queenan, K., Häsler, B., 2018. A Systems Approach to Evaluate
739   One Health Initiatives. Frontiers in Veterinary Science 5.
740 Shen, Y., Zhou, H., Xu, J., Wang, Y., Zhang, Q., Walsh, T.R., Shao, B., Wu, C., Hu, Y., Yang, L., Shen, Z., Wu, Z.,
741   Sun, Q., Ou, Y., Wang, Y., Wang, S., Wu, Y., Cai, C., Li, J., Shen, J., Zhang, R., Wang, Y., 2018.
742   Anthropogenic and environmental factors associated with high incidence of mcr-1 carriage in
743   humans across China. Nat. Microbiol., 3, 1054-1062.

744 Siokou, C., Morgan, R., Shiell, A., 2014. Group model building: a participatory approach to understanding
745   and acting on systems. Public health research & practice 25.
746 Soliman, N.F., Yacout, D.M.M., 2016. Aquaculture in Egypt: status, constraints and potentials. Aquaculture
747   International 24, 1201-1227.
748 Sommerset, I., Krossøy, B., Biering, E., Frost, P., 2005. Vaccines for fish in aquaculture. Expert Review of
749   Vaccines 4, 89-101.
750 Stentiford, G.D., Sritunyalucksana, K., Flegel, T.W., Williams, B.A.P., Withyachumnarnkul, B.,
751   Itsathitphaisarn, O., Bass, D., 2017. New Paradigms to Help Solve the Global Aquaculture Disease
752   Crisis. PLOS Pathogens 13, e1006160.
753 Tabin, J.A., Aspiras, A., Martineau, B., Riddle, M., Kowalko, J., Borowsky, R., Rohner, N., Tabin, C.J., 2018.
754   Temperature preference of cave and surface populations of Astyanax mexicanus. Developmental
755   Biology 441, 338-344.
756 Watts, J., Schreier, H., Lanska, L., Hale, M., 2017. The Rising Tide of Antimicrobial Resistance in Aquaculture:
757   Sources, Sinks and Solutions. Marine Drugs 15, 158.
758 Wernli, D., Jørgensen, P.S., Parmley, E.J., Troell, M., Majowicz, S., Harbarth, S., Léger, A., Lambraki, I.,
759   Graells, T., Henriksson, P.J.G., Carson, C., Cousins, M., Skoog Ståhlgren, G., Mohan, C.V., Simpson,
760   A.J.H., Wieland, B., Pedersen, K., Schneider, A., Chandy, S.J., Wijayathilaka, T.P., Delamare-
761   Deboutteville, J., Vila, J., Stålsby Lundborg, C., Pittet, D., 2020. Evidence for action: a One Health
762   learning platform on interventions to tackle antimicrobial resistance. The Lancet Infectious Diseases
763   https://doi.org/10.1016/S1473-3099(20)30392-3.

764 **Tables**

765 Table 1: Professions of workshop participants. Some participants represented more than one organisation.

Organisation	n
UK-based academics (from 3 institutions) *(research team)*	8
Egypt-based academics (from 4 institutions)	13
WorldFish Egypt	5
National Research Institute (ICLAR)	1
Feed factory owner	1
Farmers/fish farm owners	15
Pharmaceutical company representative/trader	1
General Organisation of Veterinary Services	2

766 **Figures**

767 Figure 1: Workshop objectives of the mapping activity, framed within four dimensions. Participants were
768 asked to consider each of the dimensions when working through each objective
769
770 Figure 2: Consensus map of the farmed tilapia production system in Egypt
771
772 Figure 3: Occupations of respondents to the post-workshop survey.
773
774 Figure 4a: Summary of responses to the post-workshop survey on the feasibility of various interventions to
775 reduce ABU and improve fish health.
776
777 Figure 4b: Summary of responses to the post-workshop survey on the advantages and disadvantages of
778 various interventions to reduce ABU and improve fish health.
779
780 Figure 5: Mean score of importance for each group of interventions (8 = highest score available and 1 =
781 lowest score available)