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9	Insight into Aquaculture's Potential of Marine Annelid Worms and Ecological
10	Concerns – A Review
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37 Abstract

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Polychaetes are marine annelid worms that can contribute to aquaculture diversification. 39 40 Its culture has been viable, and commercially attempted, but intensive production has progressed only in few countries around the world. In the countries with no production, 41 marine polychaetes are imported or harvested. A strong and sustained research 42 investment provided to a better understanding of the nutritional requirements and 43 reproduction of some species. Recent studies showed new technical improvements, 44 45 which can lead to an important progress in productivity and give a new impetus to the polychaete production. Some marine worm species were identified as good candidates 46 47 for integrated multitrophic aquaculture. The development of cost-effective aquaculture techniques for marine annelid worms is essential to ensure a balance between 48 commercial interests and the preservation of ecosystems. The influence of polychaete 49 aquaculture on the environment and vice versa raise important concerns related to 50 ecological security and sustainability of this activity. This review focus on the main 51 technical improvements and advances that have been made in areas as diverse as: 52 aquaculture potential of polychaetes, reared species, main species used worldwide, and 53 54 highlights biological and ecological important concerns, challenges and recommendations. 55

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57 Key words: Live bait, *Hediste diversicolor*, *Marphysa sanguinea*, Polychaetes

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1. Introduction

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Marine annelid worms harvesting on sediment shores is an ancient activity and has been an integral part of global coastal life. Nowadays, this activity has been widely spread, since these organisms are a highly efficient bait. The bait collection for both recreational and professional fishing purposes represents a very important business in many regions of the world (Cunha et al., 2005; Carvalho et al., 2013; Watson et al.,

2017). Several polychaetes families (such as Arenicolidae, Glyceridae, Lumbrineridae, 67 Nereididae, Nephtyidae, Onuphidae and Eunicidae) are harvested on intertidal flats, in 68 different sediment types, ranging from mud to coarse sand (Olive, 1994; Scaps, 2003; 69 Cunha et al., 2005; Cancela da Fonseca & Fidalgo e Costa, 2008; Carvalho et al., 2013; 70 Mosbahi et al., 2015; Sá et al., 2017; Font et al., 2018). 71

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In the last few years, the trade of live bait has expanded, not only due to its importance as fishing-bait, but also due to its potential as a food supply for broodstock 73 diets for aquaculture, promoting gonad maturation and spawning for a range of 74 hatchery-reared species - e.g. soles, shrimps and crabs (Luis & Ponte, 1993; Luís & 75 Passos, 1995, and references therein). Recent studies also indicated that polychaetes 76 77 could provide a nutritionally correct balance of polyunsaturated fatty acids to crustaceans and finfish, mainly of the Soleidae family, being essential for the 78 79 maturation and spawning of these species in captivity (Cardinaletti et al., 2009; Alava et al., 2017). Marine annelid worms are key prey species for aquatic organisms, including 80 81 economically important fish (e.g. sole) and invertebrates (e.g. shrimps) (Olive, 1994; Dinis et al., 1999; García-Alonso et al., 2008; Garcês & Pereira, 2011; Arias et al., 82 2013). Polychaetes are also widely used for research, as model laboratory animal, for 83 example in reproduction, ecotoxicology, bioremediation and environmental monitoring 84 studies (Fischer & Dorresteijn, 2004; Pierri et al., 2006; Cardoso et al., 2008; García-85 Alonso et al., 2008; Gray & Elliott, 2009). 86

The increasing demand for marine bait boosted the aquaculture production of 87 different marine annelid worms. Although economically viable, the current production 88 cannot supply the market demand (Fidalgo e Costa et al., 2006a; Sá et al., 2017). 89 However, bait harvesting, bait trade (Carvalho et al., 2013; Mosbahi et al., 2015) and 90 non-indigenous species imports may conflict with the marine and estuarine habitat 91 92 conservation (Font et al., 2018). Bait related activities are deficiently regulated worldwide, even though bait harvesting ecological impacts are considerable. The 93 94 existing paradigm that bait fisheries are low-value fisheries, of very limited extent, temporally intermittent, that are only ancillary to traditional fisheries, associated with 95 96 poor data availability, leads to great challenges in order to regulate and manage this 97 activity. This review aims to integrate relevant information on the state and the potential 98 rearing of polychaetes, core technical improvements and production advances, main used species worldwide and environmental concerns related to harvesting and dumping 99 100 activities.

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2. Polychaete's Potential for Aquaculture

There are a limited number of locations where marine annelid worms can be 104 105 collected in and exploited in an economically sustainable manner and without causing environmental damage. Adding to this, other concerns such as the worm's quality and 106 size, limited access to harvesting areas due to tides and seasonal variations and the 107 problems associated to imports, as introduction of non-indigenous species must be 108 109 considered. Although imports of non-native species are allowed for live wild harvested 110 bait, aquaculture of non-native species is not legally permitted in some countries, 111 including Portugal. Commercial rearing of native species is an attractive solution to 112 reduce the overcome these problems (Gambi et al., 1994; Olive, 1994; Nesto et al., 113 2012; Santos et al., 2016). As suggested by Olive (1999), this production would fulfil different needs, such as to: i) reduce indiscriminate harvesting, ii) contribute to cut 114 115 imports of non-native species, iii) promote the development of new aquaculture products, iv) enhance the species diversification in the aquaculture industry and v) 116 117 contribute to the development of new markets.

118 The environmental benefits, which may be gained from increasing bait farming and reducing bait-digging activity, are considerable. Many anglers would prefer to purchase 119 cultured bait rather than dig their own, if the supplies were of high quality and priced 120 advantageously in relation to some of the worms collected from the wild (Olive & 121 Cowin, 1994; Fowler, 1999). Furthermore, the demand for polychaetes has increased as 122 a result of their use to feed other species in aquaculture. In this way, the culture of 123 polychaetes could also provide a mean for supplying other sectors of the aquaculture 124 industry. This is especially true, when marine annelid worms are used for the 125 126 improvement of diets in hatcheries and larval rearing units, for both finfish and crustaceans. However, the production of polychaetes must be competitive in terms of 127 128 price, quality and product diversity when compared to the available wild sources, as suggested by Olive (1999). Farmers have been carrying out commercial scale trials, 129 feeding different species with reared ragworms containing special lipid and fatty acid 130 profiles (Olive, 1994), in broodstocks conditioning studies (Meunpol et al., 2005; 131 Cabrita et al., 2006; Cardinaletti et al., 2009) or to increase haematocrit and 132 haemoglobin levels in sole juveniles (Kals et al., 2015). Therefore, there is strong 133 134 evidence that polychaetes could play an important role as a protein and lipid source in fish and crustacean diets, either as a whole or as part of a compound meal, in somestages of their life cycles (Dinis et al., 1999; Meunpol et al., 2005; Alava et al., 2017).

Integrated multitrophic aquaculture (IMTA) can be a solution to improve the rearing 137 138 of marine annelid worms in some countries and at the same time contribute to reduce 139 the environmental impact of the sector. Usually, cultured organisms (as fish or shrimp) 140 need frequent feeding of artificial extruded diets. Excessive feed and faeces, deposited on the bottom of the pond, can lead to organic accumulation. In this environment, some 141 polychaetes do not require feed supplementation once they are omnivorous and able to 142 143 adapt their feeding mode, depending on food source availability (Vedel and Riisgård, 1993; Pousão Ferreira et al., 1995; Carvalho et al., 2007). Organic matter brought in 144 145 during sea water exchanges may be enough for marine biomass production and simultaneous treatment of mariculture wastewaters using sand filters, as suggested by 146 147 Palmer (2010). Nevertheless, additional feed and organic debris could be helpful to achieve a faster growth rate and maintain a higher rearing density. Therefore, 148 149 polychaetes can be cultured together with some species (that have complementary feeding habits and produce additional organic enrichment) or with the concentrated 150 151 waste sludge containing not only fish faeces but also uneaten feed and bacterial biofilms 152 (Bischoff, 2007; Fang et al., 2017). These species could include fishes, oysters, mussels, shrimps, or algae (Barrington et al. 2009; Bischoff et al., 2009; Bischoff et al., 2010; 153 Brown et al., 2011; Bergström et al., 2015). These integrated rearing systems have 154 155 environmental and economic benefits and could contribute to the sustainable growth of 156 aquaculture, based on new production technologies and systems, which increase the quality of the effluents and reduce the impacts on the environment. Marques et al. 157 (2017) have tested the integrated use of polychaete-assisted sand filters and halophyte 158 159 aquaponics for the biomitigation of a super-intensive marine fish farm effluent. The 160 integrated multi-trophic aquaculture system tested in this study showed a significant decrease of organic matter but also a substantial potential for mitigation of dissolved 161 162 inorganic nutrients (67% decrease efficiency). In the last decade of the twentieth century, scientists manifested an increasing concern regarding these impacts (Troell et 163 164 al., 1999) and more researchers have been developed their research with IMTA or 165 "Sustainable Coastal Production Systems" techniques (Newkirk, 1996; Buschman et al., 2001; Giménez Casalduero, 2001) to promote the sustainability of the aquaculture 166 production systems. 167

The growth and resulting nutritional composition of cultured Alitta virens (M. Sars, 168 169 1835), which was fed with waste from a recirculation system holding juvenile Atlantic halibut Hippoglossus hippoglossus (Linnaeus, 1758), were evaluated by Brown et al. 170 171 (2011). The presence of highly unsaturated fatty acids (HUFAS) such as DHA 172 (docosahexaenoic acid), EPA (eicosapentaenoic acid), and amino acids (AA), greatly 173 enhanced the value of these worms as components for fish or shrimp diets. The fact that these nutrients could be recovered from fish wastes offered a significant benefit in co-174 culturing these polychaetes with marine fish in recirculation systems. Plus, the authors 175 176 demonstrated that producting A. virens using fish wastes was highly effective. The 177 authors concluded that A. virens is an excellent candidate for integrated aquaculture in 178 land-based systems since it can grow rapidly on marine fish recirculation system wastes, 179 converting them into valuable biomass, which in turn may be a source of food for other 180 aquaculture species. The rearing of the polychaete Hediste diversicolor (O. F. Müller, 181 1776) fed with faeces from the carpet shell clam *Ruditapes decussatus* (Linnaeus, 1758) 182 points to similar conclusions (Batista et al., 2003a). A recent study confirmed the 183 potential of this species for the bioremediation of super-intensive marine fish farm 184 effluents and highlighted its ability to retain high value nutrients (e.g. HUFA in general 185 and EPA in particular, and to a lesser extent DHA) from fish feeds that would otherwise be lost from the production environment (Marques et al., 2018). 186

Other suggestions made by Fowler (1999) should be taken into consideration. This 187 author advises that developments in the bait species aquaculture using locally caught 188 broodstocks, also provide an important potential for the artificial restocking of the 189 populations severely depleted by overexploitation. The emergence of systems for the 190 intensive culture of these animals can provide a mean of alleviating the effects of 191 unsustainable bait collection and should reduce the need for bait imports. It is essential 192 193 that the emerging polychaetes rearing industry does not itself impose an unsustainable 194 demand on the natural environment (e.g. for broodstocks). The closed cycle aquaculture 195 of polychaetes is complete for some commercial species as A. virens but not totally controlled for other important species. Although the development of laboratory strains 196 197 is possible, aquaculture species are usually genetically different from the wild species and increase environmental concerns about possible escapes or dumping from fishing 198 199 activities. Closed cycle aquaculture of marine annelid worms can become independent of wild resources except for the occasional renewal that enhance the genetic variability 200 201 of the stocks, reducing the environmental concerns in case of escapes or introductions.

The systems developed for the culture of these worms should, therefore, include the implementation of an effective breeding protocol, which includes: broodstock rearing to alleviate the need for inputs from natural populations, procedures for mass fertilization, production of larvae and juveniles, appropriate types of new diets, optimal densities, and optimization of biomass production (Olive, 1999; Prevedelli, 1994; Prevedelli & Zunarelli Vandini, 1998; Safarik et al., 2006; Nesto et al., 2012; Santos et al., 2016).

Polychaete aquaculture has been developed for several species but there are hurdles 208 to develop this activity. Research that allowed knowledge to produce new native species 209 210 can enhance the diversification of the bait produced, develop simple inexpensive systems for rearing juveniles (García-Alonso et al., 2013), extend of breeding season 211 212 and/or cryopreservation of larvae to achieve larval supply all year round, develop appropriate breeding protocols (Nesto et al., 2018), optimize growth through control of 213 214 nutrition with new diets (Santos et al., 2016), temperature and, if appropriate, photoperiod (Olive, 1999), all contributing to improve the production of polychaetes. 215

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2.1.Farmed marine annelid worms

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219 The aquaculture of polychaete worms, in Europe, began in the United Kingdom (UK) 220 and Netherlands and has been further developed in the last few years. Farmed bait in Europe currently mainly comprises the polychaete A. virens, commonly known as 221 sandworm or king ragworm. The commercial aquaculture of A. virens was initiated in 222 223 1984 in England and, almost simultaneously, similar developments took place in the Netherlands (Olive, 1999). An UK company specialized in the production and sale of A. 224 virens, reached production values of 30 tonnes per year in the late 1990s 225 (www.ukmarinesac.org/activities/bait-collection) and became the industry leader. In 226 227 2003, an USA company and an aquaculture research center developed the world's first indoor recirculating marine polychaetes worms farm. The rearing method to produce 228 229 polychaetes can be of the intensive type, as the one adopted in the United Kingdom with A. virens, or a semi-intensive and integrated culture using residual waters from 230 231 aquaculture, which are rich in pseudofaeces and in residues. The integrated aquaculture 232 system has been adopted predominantly in Asia and Oceania for other species. This 233 method produces a reasonable quantity of biomass with reduced environmental effects (Folke et al. 1998). Applied research has also lead to significant results in various non-234 235 European Union countries, such as Australia, China and Korea (Nesto et al., 2012). In Qidong, Jiangsu (China) there is a company that produces mainly *A. virens* (Nesto et al., 2012). This reared worm is available either alive or preserved frozen. Another UK company produces, markets and develops raw materials from *A. virens* derived products which make up the oil and protein elements of aquatic animal feeds. The established industry of polychaete production has shown a sustained pattern of growth, and aquaculture has become an accepted mean of supplying the market.

In Australia, the main polychaete farm has been established since 1996. Besides *A. virens*, it produces other species, mainly *Diopatra aciculata* (Knox & Cameron, 1971). Scaps (2003) reported that *Perinereis brevicirrata* (Treadwell, 1920) is produced in Taiwan to export to Japan, and that small farms producing *Perinereis* spp. are also found in southern Japan.

247 The polychaetes production is based on adult specimens which are usually 248 collected from their natural habitat when they are mature. According to Serebiah (2015), marine annelid worms can be kept at room temperature, in aerated tanks or aquaria, 249 250 using artificial seawater. In some species, adults can be sexed according to the colour of 251 their gametogenic parapodia. The same author refers that adult male and female worms 252 are best kept in separate tanks to prevent premature spawning, although this is not easy 253 to do with all the species. Separation is easier in tube dwelling polychaetes, once a 254 longitudinal cut is quickly repaired by the worm. Larvae can be obtained naturally with males and females in the same tank (Santos et al., 2016) or with artificial fecundation 255 256 (Serebiah, 2015). Nesto et al. (2018) evaluated the effectiveness of two fertilization 257 conditions, *in vitro* and natural, using tissue homogenates or thermal shock. Percentages 258 of fertilized eggs and number of larvae were higher using in vitro fertilization, while low rates of fertilization and hatching were observed when mature individuals were 259 induced with thermal shock. These authors also suggested that male tissue homogenates 260 261 can induce females to successful spawn with high fertilization and hatching rates.

Sediment should be used in the rearing tanks to simulate the polychaetes' natural environment in large concrete tanks, other type of tanks, polyethylene containers or in aquaria for experimental trials. These systems, are used for conditioning a broodstock or to grow juveniles, with a sediment layer of 6 to 15 cm high (Nesto et al., 2012; Santos et al., 2016). Sediments should first be washed, or frozen, to kill any organisms remaining. Kaba'ah (2015) evaluated the use of the polyethylene beads as substrates for rearing *A*. *virens*. The author concluded that artificial substrate materials did not enhance culturing

and growth of this worm, compared to sand, and had significant negative effects on theproductivity.

Concerning feeding, Serebiah (2015) suggested the use of concentrated liquid
invertebrate food, diluted and broadcasted evenly over the sediment, once per week.
Commercial fish meals used daily (Santos et al., 2016) or three times per week *ad libitum* (Nesto et al., 2012) were also mentioned as diets for marine polychaetes.

Aquaculture of marine annelid worms has potential to grow since polychaetes can be produced in a sustainable way, to fulfil demand needs at a competitive price when compared to the wild animals. Overall, the aquaculture success of these worms is determined by having optimal environmental conditions, but increased human activity along the coastline (Halpern et al., 2008) can increase the cultivation's risks.

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2.2. New potential candidates

283 According to Pires et al. (2012) Diopatra neapolitana (Delle Chiaje, 1841) is the most abundant and widespread species of the genus Diopatra in Ria de Aveiro 284 285 (Portugal) but Diopatra marocensis (Paxton, Fadlaoui & Lechapt, 1995) also has a 286 well-established population at this estuarine system. These authors studied the reproductive cycle of this D. marocensis population and concluded that D. marocensis 287 is the biggest species with direct development, with the highest number of eggs and 288 larvae inside the parental tube. This species reproduces along the whole year, being the 289 290 main reproductive period extending from April to September. This characteristic, 291 associated with its high commercial value, makes D. marocensis a candidate with high 292 potential for aquaculture purposes.

Giangrande et al. (2014) evaluated the growth of the polychaete Sabella 293 294 spallanzanii (Gmelin, 1791) in rearing experiences with the purpose of using the 295 biomass as a dietary supplement for fish nourishment. The growth of this species was 296 measured in suspended plastic nets, in two sites with different trophic features, where the specimens were co-cultured with *Mytilus galloprovincialis* (Lamarck, 1819) within 297 298 a long-line. The authors demonstrated the possibility of obtaining high-value worm biomass coupled with mussel biomass, in a completely non-fed culturing system, 299 300 without increasing (presumably even reducing) the environmental impact. The polychaete Perinereis cf. nuntia (Lamarck, 1818) is a tropical endemic species in 301 302 Thailand that was also cultured in captivity (Poltana et al., 2007). In addition, various

studies and private companies have focused their attention on the onuphid *D. neapolitana* (Conti & Massa, 1998; De Murtas et al., 2003), the common ragworm - the
nereidid *H. diversicolor* (Fidalgo e Costa et al., 2000; Batista et al., 2003a and 2003b;
Masala & Piergallini, 2007; Nesto et al., 2012; Santos et al., 2016) and the rockworm the eunicid *Marphysa sanguinea* (Montagu, 1813) (Prevedelli, 1994; Garcês & Pereira,
2011; França et al., 2016).

A strong collaboration between the scientific sector and the aquaculture industry is fundamental to develop polychaete production, since the full understanding and replication of the polychaetes life cycle in aquaculture, nutritional and zootechnical requirements are central questions for a competitive commercial activity. Advances and the potential rearing of two promising species, *Hediste diversicolor* and *Marphysa sanguinea*, are detailed in the following sections.

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- 2.2.1. Hediste diversicolor (O. F. Müller, 1776)
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319 The ragworm *H. diversicolor* (O. F. Müller, 1776) occurs in estuaries and lagoons 320 located along the Atlantic coasts of North America and Europe and it has been 321 commonly used as bait and as food for fish and crustaceans reared in aquaculture. H. diversicolor is widely used as sea angling bait in Italy (Gambi et al., 1994; Nesto et al., 322 2012), France (Scaps, 2002) and along the coast of Portugal (Fidalgo e Costa et al., 323 2006a; Carvalho et al., 2013; Sá et al., 2017). It can grow and reproduce in different 324 types of sediments, since it is a semelparous species, with a high physiological tolerance 325 to a wide range of environmental factors, such as salinity, temperature and dissolved 326 oxygen (Fidalgo e Costa et al., 1998). In laboratory conditions, this worm can use 327 328 different types of food, including a suspension of clam faeces, extruded diets, semi wet pellets or fish fillet (Fidalgo e Costa et al., 2000; Batista et al., 2003a and 2003b; Santos 329 330 et al., 2014; Santos et al., 2016). Thus, *H. diversicolor* might be a promising species to be commercially exploited in indoor farming systems, with Recirculated Aquaculture 331 332 System (RAS) or in IMTA with fish or shellfish productions.

Batista et al. (2003b) have shown that the optimization of the feeding rate of *H*. *diversicolor* may cause an early sexual maturation, leading to the production of fast growing organisms that breed at smaller size. Increased juvenile growth rate may affect the trade-off curve, which relates fecundity and the development period for ectothermic

organisms (Sibly & Atkinson, 1994; Atkinson & Sibly, 1997), including nereidid 337 338 polychaetes (Olive et al., 1997; Last & Olive, 1999; Prevedelli & Cassai, 2001). In the study performed by Batista et al. (2003b) the polychaetes were fed with a commercial 339 340 dry diet developed for gilthead seabream *Sparus auratus*, and with a diet for ornamental 341 fish. Notwithstanding, Machado et al. (2016) tried to reduce the lipid content of diets for sea bream and ornamental fish. They observed that a diet with high protein content 342 (60%) and low lipid content (2%) could slow down the process of early maturation in 343 the ragworm *H*. diversicolor. However, since this kind of diet can be expensive and new 344 345 formulations or alternatives that ensure high protein and low lipid content should be considered. 346

347 The potential use of ragworms in aquaculture was suggested to reduce the organic matter of effluents and increase the reproductive fitness of reared animals, in the study 348 performed by García-Alonso et al. (2008). Aquaculture produces huge amounts of 349 sludge that can be ingested and recycled by *H. diversicolor*, maintaining their level of 350 351 ω -3 polyunsaturated fatty acids, when compared to ragworms fed with commercial fish food or eel sludge. This can be useful in order to decrease the consumption of fish meal 352 353 in the aquaculture industry. Batista et al. (2003a) conducted a study with H. diversicolor 354 fed on faeces of the carpet shell clam Ruditapes decussatus during 65 days and observed that worms were able to grow using the clam faeces, in the presence or 355 absence of sediment. However, the worms from the trials with sediment showed a 356 357 higher survival rate and greater biomass production than those from the trials without sediment. These findings suggest that a suspension of bivalve faeces can be used for H. 358 359 diversicolor rearing in integrated polyculture systems. Carvalho et al. (2007) highlight the reduction of organic matter content in ponds in which H. diversicolor have been 360 introduced has a major result of their work, most likely related to the activity of this 361 362 species on the sediment reworking. Bergström et al. (2015) examined the potential use of burrowing polychaetes in remediation efforts on sediment organically enriched by 363 364 mussel aquaculture. Their results showed that the decomposition of freshly produced mussel faecal pellets was enhanced by the presence of *H. diversicolor*, either by direct 365 366 consumption of the faeces or by bioturbation activity. Oxygen conditions are significantly improved after the addition of polychaetes at naturally occurring densities. 367 However, these authors considered that further technological developments are needed 368 in order to allow this approach to be used by the industry. 369

It is worthy to note that high-protein diets (commercial fish meals) allow higher 370 growth rates. Nesto et al. (2012) reported that both growth and survival rates were 371 higher at the lowest densities (300 ind.m^{-2}) , whereas the processes of gametogenesis and 372 sexual maturation were not influenced by density. According to the same authors, the 373 374 choice of appropriate diet formulations and the modulation of stocking densities are of great importance to commercial sized organisms, within 3.5 - 4 months from hatching. 375 Organisms maintained at a density of 1000 ind.m^{-2} and fed with high-protein food (66% 376 protein) showed the highest values of daily biomass production. However, this kind of 377 378 diet has also shown to start out earlier gametogenesis processes and earlier sexual maturity stages. 379

380 Bagarrão et al. (2014) evaluated the effect of salinity on juvenile's growth and determined the lipid composition of these organisms at different salinities. The fatty 381 382 acid profile is an important characteristic when these polychaetes are introduced in aquaculture diets for fish species, like broodstocks collections. The experiment was 383 384 conducted in a period of 60 days, during which three different salinities were tested (15, 20, and 25 psu). The individuals reached specific growth rates between 6.76% and 385 386 7.01%, and daily growth rate and final weight were higher in individuals submitted to salinities of 20 and 25 psu (p < 0.05), respectively. With this study, Bagarrão et al. 387 (2014) concluded that a salinity of 20 psu may be the most suitable for *H. diversicolor* 388 growth. Regarding the lipid profile, these authors concluded that salinity does not 389 390 influence the fatty acid composition of this species. Therefore, it is expectable that the rearing of these organisms can be carried out at different locations in coastal zones, 391 392 without changes in lipid composition.

There are still few studies regarding the fatty acids abundance and profile in 393 ragworms. Luís & Passos (1995) investigated seasonal changes in lipid profiles and 394 395 composition of wild H. diversicolor. Fidalgo e Costa et al. (2000) determined the 396 growth and survival rates of juvenile common ragworms fed with six different diets in 397 laboratory; each of them was analysed for organic matter, caloric and total lipid content and the fatty acid content of the worms was related to the specific diet. It is worthy of 398 399 note that higher levels of DHA and EPA allow a higher incorporation of HUFA, which results in higher survival rates and better growth of worms. Santos et al. (2016) studied 400 401 the effect of different diets on the growth of *H. diversicolor* juveniles, as well as the fat content, fatty acid profile, and protein content in their tissues, aiming to find an 402 403 appropriate diet to be used in commercial aquaculture. Juveniles fed with commercial

diet for seabream, had the highest final individual weight $(0.89 \pm 0.10 \text{ g})$ and the highest 404 405 daily growth rate. This study showed that the total fat content of the diets was reflected in the fat content of the reared worms. Individuals fed with a commercial diet for sole 406 407 had the highest fat content (2.25%), the ones fed with seabream dry feed showed similar 408 results (2.18%) and the lowest percentage was observed with the mackerel diet (0.85%). Regarding the fatty acid profile, the major fatty acids found in juveniles fed with the 409 three different diets were palmitic acid (C 16:0), with a higher value in the individuals 410 fed with mackerel's fillets. Oleic (C 18:1 n9), eicosapentaenoic (C 20:5 n3), 411 412 docosahexaenoic (C 22:6 n3) and stearic (C 18:0) acids were also higher in H. diversicolor fed with experimental diets. Marques et al. (2018) showed that H. 413 414 diversicolor enables the recovery of HUFAs (e.g. EPA, DHA and AA) and palmitic acid into the tissues of these polychaetes. These FA can be reintroduced into productive 415 416 systems through the potential of *H. diversicolor* to recycle these key ingredients 417 available in different food sources originating from cultivated fish.

418 According to the previous studies, the use of *H. diversicolor* can possibly reduce the production of waste and be produced in integrated multitrophic recirculation 419 420 systems. This species is able to switch its feeding behaviour (Scaps, 2002; Fidalgo e 421 Costa et al., 2006b) and, thus, fish faeces, uneaten fish food and sediment borne bacteria 422 are possible sources of nutrients to the worms. Investigation regarding the type of systems, new diets and "waste" resources is needed. In spite of all this research 423 424 development in the last decades, H. diversicolor has not been adopted as a commercial species. A solution to promote the growth and biomass increase and, at the same time, 425 delay the maturation is crucial to improve this species production in aquaculture, since 426 this species is semelparous, with a single reproductive event before the death of the 427 mature worms. 428

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2.2.2. Marphysa sanguinea (Montagu, 1813)

Prevedelli et al. (2007) studied a population of *M. sanguinea* y in Italy and found that the first phases of larval development took place in the maternal tube, which constitutes the only protection system for the eggs. Larvae (trochophores) reach the surface and continue their development after they have a complete ciliature and the ocular spots become light-sensitive due to strong positive phototropism. Larvae are not

438 very mobile, and the pelagic phase is very short, which generates an early need for439 substrate material.

Garcês & Pereira (2011) investigated the influence of salinity on the survival and growth rates of *M. sanguinea* juveniles. Their results showed that salinity changes produced an immediate and significant effect on growth. These authors recommended a salinity range between 25–35 psu, since the growth rates decreased beyond this interval. Salinities close to 15 or 40 psu seemed to be the lower and upper physiological limits, respectively, for this species.

Parandavar et al. (2015) evaluated three different densities of rockworm (500, 1000 and 2000 ind.m⁻²) and showed that the *M. sanguinea* specific growth rates decreased with increasing densities. Nevertheless, daily biomass production in different density groups can vary between 6.28 g.m⁻².day⁻¹ and 14.7 g.m⁻².day⁻¹, suggesting that this can be one of the most suitable species to be commercially exploited in a farming system. However, its production requires knowledge on reproduction and nutrition in order to develop protocols for the reproduction and appropriate rearing techniques.

França et al. (2016) analysed the influence of temperature and diet on the reproduction of *M. sanguinea*. Their results showed that a temperature range of 22 ± 1 °C and a diet with high protein levels (plus a low lipid content) originated a significantly higher density of new generation individuals than the other condition used (18 ± 1 °C and a diet with lower protein content).

Further research is needed, mainly in concerning the reproduction and nutrition of *M. sanguinea* and also regarding the density and growth potential with new diets. This research should provide support to understand if this species is suitable for a commercial exploitation in indoor or outdoor farming systems.

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3. Main species used worldwide

There are several worm species that are specially used by sea anglers worldwide (Table 1). Some of those species are mainly supplied by Korea, China, Vietnam and USA, the major international market suppliers nowadays (Fidalgo e Costa et al., 2006a; Arias et al., 2013; Sá et al., 2017; Font et al., 2018).

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471 Table 1 – List of the worm species that are mainly used by sea anglers worldwide.

Scientific name	Scientific authority	Common or trade name(s)
Alitta succinea	Leuckart, 1847	Pile worm, clam worm
Allita virens	M. Sars, 1835	Sandworm, king ragworm
Arenicola defodiens	Cadman & Nelson-Smith, 1993	Black lugworm
Arenicola marina	Linnaeus, 1758	Lugworm, blow lugworm
Arenicolides branchialis	Audouin & Milne Edwards, 1833	_
	Edwards, 1855	-
Arenicolides ecaudata	Johnston, 1835	Tail-less lugworm
Diopatra aciculata	Knox & Cameron, 1971	Tube worm
Diopatra cuprea	Bosc, 1802	Plumed worm
Diopatra neapolitana	Delle Chiaje, 1841	Solitary tube worm, casulo
Glycera americana	Leidy, 1855	American bloodworm
Glycera	Ehlers, 1868	American bloodworm,
dibranchiata		common bloodworm
Halla parthenopeia	(Delle Chiaje, 1828)	Llobarrero, minhocão
Hediste diversicolor		Common ragworm, mud
	O.F. Müller, 1776	worm, minhoca-da-pesca,
		tremolina
Marphysa	Mostory 1915	Deel
sanguinea	Montagu, 1815	Rockworm, ganso
Namalycastis	Glasby, Miura, Nishi &	Nypa pal worn, nuclear worm,
rhodochorde	Junardi, 2007	magic cord
Nephtys caeca	Fabricius, 1780	Large white ragworm, silvers
Nephtys cirrosa	Ehlers, 1868	White catworm

Nephtys hombergii	Savigny in Lamarck, 1818	Catworm
Nereis limbata	Ehlers, 1868	Cinder worm
Nereis pelagica	Linnaeus, 1758	Slender ragworm, red ragworm
Perinereis nuntia vallata	Grube, 1857	-
Perinereis aibuhitensis	Grube, 1878	Korean lugworm, Korean blue ragworm
Perinereis cultrifera Perinereis linea Perinereis nuntia	Grube, 1840	Ragworm, green worm
	Treadwell, 1936	Korean ragworm
	Savigny in Lamarck, 1818	-
Platynereis dumerilii	Audouin & Milne Edwards, 1834	Dumeril's clam worm
Scoletoma impatiens	(Claparède, 1868)	Teagem

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There is currently a high market demand for bait, which is sold with high prices. For 474 example, in the four most important Portuguese brackish water systems (Ria de Aveiro, 475 Ria Formosa lagoons, Tagus and Sado estuaries) bait harvest yield an average of 4 476 million euros per year (unpublished data). In Canal de Mira (Ria de Aveiro, Portugal), 477 the annual harvest of *D. neapolitana* exceeds 45 tonnes and is evaluated at over 325 000 478 € per year (Cunha et al., 2005). In 2003, a total of 16.87 million polychaetes were 479 imported to Europe from China and USA, via Lisbon Airport, with a worth of 716 180 480 and 291 845 US \$, respectively (Fidalgo e Costa et al., 2006a). Although recent data 481 show a decrease on these importation numbers, they are still relevant with an annual 482 mean value of 14.24 tonnes on the 2012–2015 period (Sá et al., 2017). Watson et al. 483 (2017) estimated that 1600 tonnes of A. virens per year (worth £ 52 million) are landed 484 in the UK, with approximately 121 000 tonnes of polychaetes collected globally, valued 485 at £ 5.9 billion. These authors considered that these values are comparable to many of 486 the world's most important fisheries. However, this data also proves that the current 487

landings records for marine annelid worms are underestimated: The National Marine 488 489 Fisheries Service (2015) recorded only 290 tonnes landed in 2014 for the USA (Watson 490 et al. (2017) estimate a median of nearly 19 000 tonnes), whilst the FAO database has a global mean of only 439 ± 23.7 tonnes per annum from 2000-2012 (FAO 2014). 491 492 According to Watson et al. (2017) it would be impossible to provide a direct global 493 assessment of all bait collection with the resources and management frameworks available to fisheries agencies. However, where ancillary data are available, local data 494 can be scaled resulting in the first assessments. The lack of reliable data and the non-495 496 existent fishery estimates leads to a highly significant gap in conservation management.

Many marketed polychaetes are caught from the wild or come from imports. Both activities carry negative impacts (e.g. risk of introduction of alien species, disruption of ecosystems), which in turn contribute to the growing interest of rearing these organisms. Clearly, not all the mainly used polychaetes could be produced in aquaculture, but advances with some potential species may contribute to the reduction of those negative impacts.

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4. Ecological concerns

4.1. Bait importing and dumping

Importation and trade of non-indigenous worms are special concerning subjects, 508 since they are sold as live bait worldwide (Font et al., 2018). Also, baitworms have been 509 described as potentially invasive organisms. According to Çinar (2013), a total of 292 510 polychaete species, belonging to 164 genera and 39 families, have been transported 511 512 around the world's oceans with human-mediated assistance. Some species imported and 513 exported as fishing bait became established at non-native locations. The Mediterranean Sea (134 species), the coasts of the Hawaii Islands (47 species) and the Pacific coasts of 514 515 USA (34 species) have been invaded by non-indigenous species of worms. This kind of introduction can be accidental or intentional. However, if they become dominant in their 516 new environment, these organisms are referred to as "invasive" (NISC, 2006). For 517 518 instance, Arias et al. (2013) reported that in the last few decades, the rate of biological 519 invasion has increased in the Mediterranean Sea, leading to significant ecological and economic impacts. The risks associated to the introduction of non-indigenous species 520 521 include, on one hand, the economic and ecological threatening of the coastal marine

ecosystems well-being, and, on the other hand, the simultaneous transport of other 522 523 associated hitchhiker species, which may act as vectors for parasites and diseases 524 (Elliott, 2003; Çinar, 2013). Examples of other species that can be inadvertently inside 525 the package are seaweeds and other organisms contained within or attached to it 526 (Weigle et al., 2005; Yarish et al., 2009; Cohen, 2012; Haska et al., 2012; Arias et al., 527 2013). These organisms may impact the invaded ecosystem, extinguishing endangered species or causing mass mortality of native host; they are also a risk to public health 528 (Cohen et al., 2001; Arias et al., 2013). The international trade of live bait entails 529 530 similar risks to those caused by the ballast water of ships in international traffic, being 531 responsible for most of the accidental introductions of non-indigenous worm species 532 worldwide (Fidalgo e Costa et al., 2006a; Sá et al., 2017). Fidalgo e Costa et al. (2006a) 533 reported that the Korean blue ragworm *Perinereis linea* (Treadwell, 1936) (cited as 534 Perinereis aibuhitensis (Grube, 1878) has been successfully reared in a closed system in captivity, within the range of the environmental conditions existing in the Ria Formosa 535 536 coastal lagoon (a Natural Park in the south of Portugal), both from wild imported specimens and from individuals born and grown in experimental circumstances. 537 538 According to the preliminary results, this species also seemed to be able to reproduce in 539 coastal lagoons and estuaries from the south of Portugal. Despite this, developments in 540 the culture of non-native species should be monitored very carefully and actively discouraged. 541

Bait operations involve several risk factors, including the large number of 542 543 imported species, lack of knowledge about the topic of marine invasions, the use of the common names instead of scientific names, and the discharge of untreated holding-tank 544 water and unused products (Weigle et al., 2005). It is known that, for years, a significant 545 percentage of recreational fishermen (approximately 40%) discarded the unused 546 547 baitworms, wrapping seaweeds and their package into the water (Lau, 1995). Thus, the probability of introducing non-indigenous species increased with this practice (Haska et 548 549 al., 2012; Saito et al., 2014; Sá et al., 2017; Font et al., 2018), especially because this likelihood is related not only to the number of individuals that are introduced (propagule 550 551 pressure), but also to the number of release events (Allendorf & Lundquist, 2003). This 552 is of special concern when considering that the numbers regarding the importation of wild bait to European countries are not well known (Fowler, 1999). Micael et al. (2016) 553 reported that only polychaete species were identified as imported live-bait at São 554 555 Miguel island (Azores, Portugal) and all specimens were identified as P. linea. In that

study, responses to surveys conducted among 77 anglers showed that 31 % disposed of 556 557 the remaining bait into the sea. Another field survey was developed at five sites where 558 fishing rods are routinely used, to investigate if the non-indigenous polychaetes sold as 559 fishing-bait were already established in the natural environment of the Azores 560 archipelago. Three species of Nereididae were identified: the native Neanthes nubila (Savigny, 1822) and *Perinereis oliveirae* (Horst, 1889), plus the cryptogenic *Perinereis* 561 cultrifera. Weigle et al. (2005) surveyed bait businesses in the Massachusetts region 562 (USA) and observed that 60% of retailers, who imported non-indigenous worm species, 563 564 received them packed with seaweed. A high percentage of these retailers noticed nontarget species within the same package, associated with the imported organisms, within 565 566 the same package. In addition, almost half of the surveyed retailers were not familiar 567 with the economic and ecological impacts of marine invasive species. Similar 568 conclusions were obtained by Font et al. (2018) to Catalonia. Contrastingly, Sá et al. (2017) examined several live bait boxes purchased at Portuguese stores and all of them 569 570 were packed with synthetic materials (e.g. polyester fibre, sponge and paper pulp), 571 sawdust and sand. The detailed observation of the packing materials indicated the 572 residual occurrence of oligochaetes (e.g. Enchytraidae). Those results showed that the 573 packing procedures of importers, such as transferring the imported species into water 574 tanks and re-packing it using inert materials, might be important to reduce the risk of introduction of hitchhiker species. 575

Recently published, the European Code of Conduct on Recreational Fishing and 576 Invasive Alien Species (Council of Europe, 2014) stated that recreational anglers must 577 'prevent the release, spreading and translocation of invasive alien species that can have 578 significant impacts on native fish populations or the environment'; also they must 'use 579 580 bait, particularly live bait, only in agreement with local or national regulations and use 581 aquatic organisms only in the water body from which these were collected' and 'never transfer aquatic live bait from one water body to another'. As usual, the applicability of 582 583 this code is very low until the different European countries have produced legal regulations to enforce it at the different national levels. 584

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4.1.1. Recommendations for prevention and control

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588 It is essential to educate local fishermen, sport anglers and retailers about the risks 589 of dumping live baitworms, fragmented or as a whole, or even their packing boxes into the sea, and how these actions may have economic and ecological impacts, especially concerning to invasive species (Arias et al., 2013). Promoting the use of native baitworms, plus alerting to the disposal of the unused worms, seaweeds and their containing boxes in the trash (and not in the water), will help to prevent and control the introduction of non-native species (Balcom & Yarish, 2009; Arias et al., 2013; Font et al., 2018).

Haska et al. (2012) proposed the development of practice guidelines, to be used 596 by wholesalers and retailers, and a system of certification, in order to introduce only 597 "invasive-free" bait worm products in the market. Olenin et al. (2011) also made some 598 recommendations, namely: conduct in situ continuous monitoring and sampling studies 599 600 to assess bio-invasion risks; create an international operation to develop criteria defining 601 which species are potentially invasive and which should not be imported and traded; 602 quantify economic losses and compare them to any eventual benefits gained with the 603 introduction of non-indigenous species; involve the general public in the monitoring and 604 report of the existence of non-indigenous species in coastal environments; and use 605 native species in aquaculture projects, whenever possible.

Furthermore, the detailed monitoring of the population dynamics of a species that was already introduced in a certain region, as well as the development of the System of Certification of Origin, with specific name, are options that will help control the problem and will surely be beneficial for the environment, economy and human health, as suggested by Arias et al. (2013).

In addition, it is necessary to implement prevention and control measures regarding the hitchhiker species. Sá et al. (2017) described the procedures adopted by the main Portuguese importer, which consists on a temporary storage of specimens in clean water tanks and their re-packing in new bait boxes with inert packing materials.

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4.2.Gathering worms from natural environment

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Usually, polychootes are herwooted from intertidal estuaring f

Usually, polychaetes are harvested from intertidal estuarine flats. To collect them,
the most used method is bait digging, which consists on manually turning the sediment
with a fork or a similar tool and removing the target organisms by hand (Fowler, 1999;
Watson et al., 2007; Sypitkowski et al., 2009; Birchenough, 2013; Fidalgo e Costa et al.,
2016; Sá et al., 2017). This activity occurs at mid to low tide, both during the night and
the day (Fearnley et al., 2013). Watson et al. (2017) estimated that collectors removed

624 on average 1.4 kg of A. virens per person per hour, walking a considerable distance 625 across the intertidal sediment, to reach areas that were usually already dug. It is also common to harvest bait using dredges (Beukema, 1995; Sypitkowski et al., 2010). Bait 626 627 dragging is a method used to collect polychaetes of the family Nereididae, in particular 628 the king ragworm A. virens (Birchenough, 2013). This activity takes place at high tide and is conducted from a boat, using hooked metal drags (Dyrynda & Lewis, 1994; 629 Fearnlev et al., 2013). The impacts of this method received considerable attention, with 630 studies investigating its effects on ecosystems (Beukema, 1995; Watson et al., 2007). 631 The disturbance can be significant: Klawe & Dickie (1957) estimated that bloodworm 632 diggers dug about 460 m^2 per tide in Nova Scotia, with a tidal amplitude that allows 633 longer digging time per tide. In Maine, bloodworm diggers typically dug about 180 m^2 634 each tide, and so an average digger digs about 90 m².h⁻¹ for roughly 2 h each tide 635 636 (Sypitkowski et al., 2009; Sypitkowski et al., 2010).

637 Harvesting activity of marine worms may affect the prey availability of target and 638 non-target species, causing direct and indirect negative effects (e.g. McLusky et al., 1983; Ambrose et al., 1998; Dias et al., 2008; Birchenough, 2013). It is well known that 639 640 commercial hand digging of baitworms can disturb the characteristics of the intertidal 641 benthos, affecting intertidal communities, crustaceans, fish, and birds. There is also 642 some evidence that digging releases nutrients and heavy metals from the sediment to the water (Howell, 1985; Milius, 1999; Shepherd & Boates, 1999; Falcão et al., 2006). The 643 644 supervision of harvesting activities, as well as the policies and concerns on sustainable control of intertidal resources do not act properly. This undefined exploitation 645 646 contributes to a parallel economy and directly conflicts with a sustainable and controlled activity. The bait exploitation in Portugal is allowed, using hand gathering or restricted 647 gear by licensed persons, but in reality there are large numbers of non-authorised 648 649 persons collecting bait (Cunha et al., 2005; Carvalho et al., 2013; Fidalgo e Costa et al., 2016; Sá et al., 2017). In Maine (USA), worms must be dug by hand and diggers must 650 651 pay a nominal fee for a license, but there is no restriction on the number of worms that can be harvested. There are about 1000 licensed marine worm diggers per year and, 652 despite this, the Maine Department of Marine Resources (DMR) does not differentiate 653 654 worm licenses by target species (Sypitkowski et al., 2010). According to Watson et al. (2007) licences are unlikely to control the level of bait collection on the shore, or the 655 656 frequency of collection visits, mainly because the variability in the frequency of visits,

time spent and the difficulties in assessing what is an appropriate level of activity for asite.

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4.2.1. Management measures

Despite the importance of polychaete fishery, which extracts significant biomass 662 and has, consequently, considerable impacts on the environment, there are few 663 regulations (e.g. Fidalgo e Costa et al. 2006a; Font et al., 2018). This means that this 664 665 activity requires urgent governance, similar to the one that controls other fisheries. A 666 considerable part of the polychaetes harvested for bait fishing comes from estuarine 667 protected areas or natural reserves. Species conservation and maintenance of ecosystem 668 functioning should be conciliated with the human interests and economic development. 669 A number of management initiatives have been suggested to reduce conflicts or mitigate the effects of bait collection on the macrobenthic communities. Spatial or temporal 670 671 zonation is often cited as a method to preserve bait stocks, reduce the impacts on 672 macrofauna and sediments, as well as a method of allowing damaged habitats to recover 673 (Watson et al. 2007). In particular, a rotating system, where different parts of the site are 674 closed, has gained support relying on the idea that these areas may act as sources of 675 recruitment for the exploited species, as suggested by Fowler (1999). Nevertheless, 676 some other macrofauna species suffer immediate impacts and may take many years to 677 recover, even if bait collection is suspended. Permanent no-harvesting zones may be the best option for preserving these species, since it is unlikely that these areas, once 678 679 subjected to bait collection, will regain the species lost. It is the continued disturbance that precludes their development. Ultimately, the ecological benefit of any management 680 681 scheme must be tested scientifically before implementation. Naturally, it must also take 682 into account a wide range of social, economic and other ecological issues to effectively manage the resource now and in the future (Watson et al., 2007). 683

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687 Future prospects

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689 The demand for wild-caught polychaetes could increase and aquaculture can 690 play a key role supplying the markets. This has already led to new studies and to the

691 emergence of a number of polychaete culture systems, advances in nutrition and 692 reproduction techniques. However, more investigation is needed to enable the 693 successful production of new marine worm species. The development of rearing 694 techniques and grow-out procedures are central points. They will allow the culture of 695 new polychaetes, possible in polyculture systems that can enhance aquaculture 696 sustainability, reducing environmental risk and increasing profits.

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