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Gonadal Maturity in Beong

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1 **THE EFFECT OF MIXED ANIMAL-VEGETABLE OILS IN FEED ON EARLY**
2 **GONADAL MATURATION OF MALE ASIAN REDTAIL CATFISH (*Hemibagrus***
3 ***nemurus*)**

4
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13
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15
16
17 **ABSTRACT**

18
19 Male broodstock candidates of Asian redbtail catfish tend to have aggressive behaviour due to
20 excessive testosterone production, which is exacerbated by poorly-regulated high-cholesterol
21 feeding practices. This study aimed to suppress these conditions by administering the dietary
22 combination of animal and plant-based oils during pre-gonadal maturation of male broodstock
23 candidates (BW: 215.83±41.28 g; BL: 272.12±19.89 mm), reared in a 2 × 1 × 1 m³ net cage
24 with the stocking density of two fish per cage. This study was conducted using a completely
25 randomized design with three dietary combination treatments (2% of each oil dosage) with four
26 replicates: base feed with no mixed dietary oil (K), mixed fish oil-maggot oil (IM), and mixed
27 fish oil-palm oil (IS). All parameters, namely reproductive performance (final length/Pt, final
28 weight/Wt, feed intake/FI, gonadosomatic index/GSI, and hepatosomatic index/HSI) and blood
29 chemistry profile (total cholesterol/TC, high-density lipoprotein/HDL, triglycerides/TG, and
30 glucose/GLU), were statistically tested their significance using the analysis of variance and
31 Duncan's multiple range test with 95% confidence level. Significant differences were found in
32 Pt and Wt ($p < 0.05$), where the dietary combination of oils (IM-IS) showed higher Pt and Wt
33 values than the dietary without the mixed oils (K). The IM treatment obtained the highest TG
34 level (159.98±38.44 mg dL⁻¹; $p < 0.05$), yet showed no significant differences from the other
35 treatments in TC, HDL, and GLU levels ($p > 0.05$). This study concludes that the dietary
36 combination of fish oil (2%)-maggot oil (2%) improves lipid deposition during pre-gonadal
37 maturation and regulates energy storage through weight gain, without jeopardizing the somatic
38 growth (length) of male Asian redbtail catfish broodstock candidates.

39
40 **KEYWORDS:** broodstock; catfish; diets; oil; pre-maturation

41
42 **ABSTRAK:** Pengaruh Kombinasi Minyak Hewani dan Nabati dalam Pakan terhadap
43 **Pematangan Gonad Awal Ikan Baung (*Hemibagrus nemurus*) Jantan**

44
45 *Calon induk ikan baung jantan memiliki sifat agresif yang salah satunya disebabkan produksi*
46 *testosteron berlebih akibat pemberian pakan dengan kadar tinggi kolesterol. Penelitian ini*
47 *ditujukan untuk mengurangi dampak tersebut melalui evaluasi kombinasi minyak hewani dan*
48 *nabati pada pakan selama fase awal kematangan gonad calon induk ikan baung jantan (BW:*
49 *215,83±41,28 g; BL: 272,12±19,89 mm), yang dipelihara pada hapa ukuran 2 × 1 × 1 m³ dan*
50 *padat tebar sebesar dua ekor ikan per hapa. Penelitian ini menggunakan rancangan acak*
51 *lengkap dengan tiga perlakuan pakan kombinasi (dosis tiap minyak 2%) dan empat ulangan,*

52 yaitu pakan tanpa kombinasi (K), pakan dengan kombinasi minyak ikan-maggot (IM), dan
53 minyak ikan-sawit (IS). Seluruh parameter, yaitu kinerja reproduksi (panjang akhir/Pt, bobot
54 akhir/Wt, total konsumsi pakan/FI, indeks somatik gonad /GSI, dan indeks somatik hati /HSI)
55 dan profil kimia darah (total kolesterol/TC, high-density lipoprotein/HDL, trigliserida/TG, dan
56 glukosa/GLU), dianalisis menggunakan analisis sidik ragam dan uji jarak berganda Duncan
57 dengan derajat kepercayaan 95%. Perbedaan nyata ditunjukkan pada Pt dan Wt ($p < 0,05$),
58 dimana kombinasi minyak IM-IS menunjukkan nilai Pt dan Wt yang lebih tinggi dibandingkan
59 tanpa pemberian kombinasi minyak (K). Perlakuan IM memperoleh konsentrasi TG tertinggi
60 ($159,98 \pm 38,44 \text{ mg dL}^{-1}$; $p < 0,05$), namun menunjukkan tidak adanya perbedaan nyata terhadap
61 perlakuan lainnya pada konsentrasi TC, HDL, dan GLU. Penelitian ini menyimpulkan, bahwa
62 kombinasi minyak ikan (2%) dan minyak maggot (2%) mampu memperbaiki penyimpanan
63 lemak untuk fase awal kematangan gonad (trigliserida dalam serum darah) dan mendorong
64 penyimpanan energi melalui peningkatan bobot, tanpa mengganggu pertumbuhan somatik
65 (panjang) dari calon induk ikan baung jantan.

66

67 **KATA KUNCI:** ikan baung; induk; kematangan gonad; minyak; pakan

68

69

70 INTRODUCTION

71 Asian redbtail catfish (*Hemibagrus nemurus*) is a freshwater fish species commonly found
72 in Sumatra, Java, and Borneo Islands, Indonesia (Kusdiarti *et al.*, 2020). The fish has been
73 farmed throughout Indonesia, particularly in Central Java, as a viable response to its declining
74 wild population (Mujtahidah *et al.*, 2021; Yuanawati *et al.*, 2022). For example, in Magelang,
75 Central Java, farming activities of this species not only improve its wild stock but also supply
76 local demand for high-quality animal protein. Despite the positive developments, the
77 consumption rate of this fish remains at 3.6% of total fish production, whether from fishing or
78 aquaculture activities (Ikhsanudin *et al.*, 2023).

79 The low consumption rate of Asian redbtail catfish could be attributed to limited
80 production and inconsistent seed supply, due to unsuccessful breeding activities, low fertility
81 (51%) and hatching rate (43%) of the fish eggs (Rasyad *et al.*, 2023). Since they are mostly
82 sourced from wild-caught populations with no intensive selection, Asian redbtail catfish
83 broodstock candidates, specifically male fish, are susceptible to stress and exposure to low-
84 quality diets, which reduces their gonadal maturation capability (Prianto *et al.*, 2015). Male
85 Asian redbtail catfish are also more aggressive than female fish, due to high testosterone

86 production, which can lead to cannibalism with an incidence of up to 30% and affect the pre-
87 gonadal maturation process (Heltonika *et al.*, 2023). High testosterone concentrations in
88 broodstock can be caused by exposure to uncontrolled cholesterol levels due to poor feeding
89 practices, namely, feeding the fish with trash fish during the pre-gonadal maturation period
90 (De-Dios *et al.*, 2022). Dietary combined supplementation with animal- and plant-based oils
91 could remediate these issues in male Asian redbtail catfish broodstock candidates, particularly
92 during the pre-gonadal maturation period.

93 Previously, oils, either plant-based or animal-based, have been used to support gonadal
94 maturation in various broodstock fish. Sattang *et al.* (2021) reported that 1-2% fish oil in diets
95 improved spermatocyte production and accelerated the spawning process in hybrid catfish
96 (*Pangasius larnaudii* × *Pangasianodon hypophthalmus*, Sauvage, 1878). Furthermore, fish oil
97 (60%), combined with plant-based oil, such as linseed oil (40%), could increase the weight
98 gain, gonadosomatic index, and spawning frequency of Gilthead seabream (*Sparus aurata*)
99 (Izquierdo *et al.*, 2015; Turkmen *et al.*, 2017; Ferosekhan *et al.*, 2020). The use of palm oil
100 (50%) + rubber seed oil (50%) had resulted the highest feed intake and final weight, while
101 minimizing stress condition on giant gourami (*Osphronemus goramy*) (Purnamasari *et al.*,
102 2020) and striped catfish (*Pangasianodon hypophthalmus*) (Fatmawaty *et al.*, 2021), based on
103 elevated high-density lipoprotein and low total cholesterol levels. In Asian redbtail catfish,
104 applying fish oil and corn oil (1-2% for each oil) obtained gonadosomatic index value between
105 16-16.7% (Suhenda *et al.*, 2009).

106 Another animal-based oil, extracted from black soldier fly (BSF) maggot, also has the
107 potential to improve reproductive performance and reduce stress in broodstock candidates.
108 Maggot oil has been applied as a dietary supplement to support fish and shrimp growth at 1.5-
109 1.9% (Herawati *et al.*, 2022; Herawati *et al.*, 2023). Maggot oil also contains arachidonic acid
110 (20:4n-6; 0.1%) and eicosapentaenoic acid (20:5n-3; 0.15%) as main builders of prostaglandin

111 hormone to accelerate gonadal maturation (Sattang *et al.*, 2021; Afriani *et al.*, 2023). However,
112 studies on the application of maggot oil, or its combination with fish oil, in dietary
113 supplementation to support gonadal maturation in broodstock candidates remain limited.

31 114 Therefore, this study was conducted to determine the effects of combining plant-based
44 115 and animal-based oils in the diets of male Asian redbtail catfish broodstock candidates during
116 the pre-gonadal maturation period. This study was also aimed at improving reproductive
117 performance and nutrient deposition (lipid/cholesterol) in male broodstock candidates before
118 broodstock selection for spawning process preparation, by improving body weight,
119 gonadosomatic index, hepatosomatic index, and a stress reduction indicator (cholesterol
120 concentration).

121

19 122 **MATERIALS AND METHODS**

123 **Location and Period**

124 This study was performed in July – September 2025 at the Operational Unit of Freshwater
125 Fish Culture and Hatchery (BPBIAT), Ngrajek, Magelang, for broodstock candidate rearing
126 and the Laboratory of Animal Nutrition, Universitas Tidar, Magelang, for blood chemical
127 profiling of male Asian redbtail catfish broodstock candidates.

128

129 **Ethical Statement**

15 130 This study was conducted in accordance with the established guidelines for the
131 treatment of experimental animals and fully complied with the ethical code governing animal
132 experimentation (SNI 8228-4:2022).

133

10 134 **Experimental Design**

135 This study employed an experimental method using a completely randomized design
136 with three diet treatments in four replications: combining fish oil and maggot oil (IM), fish oil
137 and palm oil (IS), and no-oil combination (control/K). The combinations of plant and animal
138 oils were applied at a 1:1 ratio, with each oil dosed at 2% (Purnamasari *et al.*, 2020; Sattang *et*
139 *al.*, 2021; Afriani *et al.*, 2023). In total, there were 12 experimental units, following the number
140 of treatments and replications.

141

142 **Broodstock Candidate and Rearing Media Preparation**

143 The 24 male fish of Asian redbtail catfish (body weight/BW: 215.83 ± 41.28 g; body
144 length/BL: 272.12 ± 19.89 mm) were obtained from BPBIAT, Ngrajek, Magelang. These male
145 fish had been reared for 6-8 months and were validated as broodstock candidates, visually
146 pathogen-free (no red/white spots and no parasite attachments), physically intact, and swam
147 actively. The rearing medium used a $2 \times 1 \times 1$ m³ net cage, installed in a $20 \times 10 \times 1.5$ m³ pond.
148 Before stocking into the net cage at 2 fish per cage, the fish were weighed per experimental
149 unit (cage) to determine the initial body weight and ensure weight uniformity. After being
150 fasted for 24 hours, fish were fed with the treatment diets for 30 days.

151

152 **Diet Preparation and Feeding**

153 Diets, containing commercial feed (*STP KAE-starter*, PT. Suri Tani Pemuka, Japfa
154 Group, Indonesia; protein 50-52%; lipid 10-11%) and oil combinations (K, IM, IS), were
155 manufactured through a coating method and dried at 60-80°C for 3-4 hours. After drying, all
156 diets were stored in dried and sealed container. Feeding practice was performed until apparent
157 satiation twice a day, namely in the morning (08.00 WIB) and in the afternoon (16.00 WIB).
158 Before and after feeding, all diets were weighed to obtain total feed intake (FI) from the first
159 to the last feeding period (30 days).

160

161 **Harvesting (Final Sampling)**

162 All fish were harvested after 30 days of rearing for further gonadal maturation
163 (broodstock selection). Before harvesting, the fish were fasted for 24 hours, then weighed and
164 measured individually to obtain their final weight (Wt) and length (Pt). Blood samples were
165 collected from each fish using a 1-ml syringe. After blood collection, the fish were euthanized
166 to collect and measure the weight of their gonad and liver to determine the gonadosomatic
167 index (GSI) and hepatosomatic index (HSI), respectively.

168

169 **Blood Chemistry Profiles**

170 Blood samples from three randomly selected fish were centrifuged at 5,000 rpm for 10
171 minutes to obtain plasma. These samples were determined to have their chemistry profiles,
172 namely total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG), and glucose
173 (GLU). The blood chemistry profiling was determined using *Human GmbH blood test kit*
174 (*Human Diagnostics Asia Pacific, Pte., Ltd., Singapore*).

175

176 **Water Quality**

177 During the rearing period, water quality parameters were maintained at 29.25-29.71°C,
178 pH 8.36-8.53, dissolved oxygen 7.24-7.77 mg L⁻¹, and ammonia 0.01-0.02 mg L⁻¹, following
179 Setiadi *et al.* (2022).

180

181 **Data Analysis**

182 Data analysis was performed using *Microsoft Excel 2024 (Microsoft Inc., USA)* and *SPSS*
183 *16.0 (IBM Inc., USA)*. All measured parameters, namely FI, Wt, Pt, GSI, HSI, TC, TG, HDL,
184 and GLU, were tested for homogeneity and normality using the Shapiro-Wilk and

185 Kolmogorov-Smirnov tests. Data that met the criteria for homogeneity and normality were
 186 further analyzed using one-way analysis of variance (One-way ANOVA) at a 95% confidence
 187 level. If significant differences were found ($p < 0.05$), analysis was continued using Duncan's
 188 multiple range test (DMRT) to determine the most effective diet to improve reproductive
 189 performance and blood chemistry profiles of male Asian redbtail catfish broodstock candidates.

190

191 RESULTS AND DISCUSSION

192 Reproductive Performance

193 The reproductive performance in this study comprises several parameters: feed intake
 194 (FI), final length (Pt), final weight (Wt), gonadosomatic index (GSI), and hepatosomatic index
 195 (HSI). The reproductive performance results of male Asian redbtail catfish broodstock
 196 candidates are presented in Table 1.

197 Table 1. Reproductive performance of male Asian redbtail catfish broodstock candidates, fed
 198 with combined oil-supplemented diets

Parameters	K	IM	IS
FI (g)	173.25±32.12	132±72.75	104.5±17.90
Pt (mm)	263.68±2.45 ^a	290.25±7.76 ^{ab}	305.75±31.34 ^b
Wt (g)	156±0.00 ^a	230.5±32.28 ^b	264.75±68.63 ^b
GSI (%)	0.0045±0.00	0.0037±0.0023	0.0049±0.0027
HSI (%)	0.0097±0.00	0.0095±0.0015	0.0085±0.0005

199 Note: All values are presented in average ± standard deviation (SD). Different superscript letters in the same line
 200 indicate significantly different values ($p < 0.05$). K = Supplementation of oil combination absence
 201 (0%/control), IM = Dietary supplementation of fish oil (2%) and maggot oil (2%), IS = Dietary
 202 supplementation of fish oil (2%) and palm oil (2%). FI = feed intake; Pt = Final length; Wt = Final weight;
 203 GSI = Gonadosomatic index; HSI = Hepatosomatic index.

204 In reproductive performance, all parameters indicate non-significant differences ($p > 0.05$),
 205 except in Pt (final length) and Wt (final weight). The final length and weight of male broodstock
 206 candidates fed different supplemented diets (IM and IS) were higher than those in the control
 207 diet (K), yet no significant difference was found in the FI parameter ($p > 0.05$). This condition
 208 means that supplying animal-based oils (fish and maggot oil) or animal- and plant-based oils
 209 provides more efficient growth (somatic and tissue improvements) and more energy deposit,

210 rather than energy for catabolism or physiological maintenance, resulting in low nutrient intake
211 (e.g. feed intake/FI) to support the reproduction process (Villamarín *et al.*, 2016; Liu *et al.*,
212 2022), specifically the spermatogenesis process in male fish. Similar results were also reported
213 by Ayisi *et al.* (2018) in Nile tilapia (*Oreochromis niloticus*), who applied palm oil and fish oil,
214 and Kimou *et al.* (2023) in African catfish (*Heterobranchus longifilis*), who combined fish oil
215 and maggot oil.

216 Despite showing a promising association among Wt, Pt, and FI, the supplemented-diet
217 treatments had no effect on GSI and HSI values ($p>0.05$). However, this result confirms that
218 male Asian redbtail catfish broodstock candidates, as in spotted scat (*Scatophagus argus*), start
219 to allocate extra metabolizable energy to reproduction (gamete production), while the gonad
220 and liver remain at constant weight during pre-gonadal maturation (Wang *et al.*, 2022).
221 Furthermore, liver and gonad are main organs in supplying energy for the reproduction process
222 (spermatogenesis) (Zhou *et al.*, 2022), thus both organs in male Asian redbtail catfish broodstock
223 candidates fed with animal- and plant-based oils (IM and IS diets) may only change their
224 nutrient component deposition, like lipids and glycogens, during pre-gonadal maturation.
225 Another study reported by Suhenda *et al.* (2009) also showed no significant difference in GSI,
226 when female Asian redbtail catfish broodstocks were fed with fish oil and corn oil-supplemented
227 diets under 1-4% dosage of each oil. In addition, other fish, such as mahseer (*Tor tambroides*)
228 broodstock fed with fish oil and corn oil at a 1:1 ratio (Abduh *et al.*, 2021) and hybrid catfish
229 (*Pangasius larnaudii* × *Pangasianodon hypophthalmus*, Sauvage, 1878) broodstock fed with
230 1-2% fish oil-supplemented diets (Sattang *et al.*, 2021), obtained no significant difference in
231 GSI and HSI.

232 The non-significant differences found in almost all parameters (FI, GSI, and HSI)
233 indicate a longer rearing time for feeding the supplemented diets, namely 107-117 days (Utiah
234 *et al.*, 2007), and much older broodstock candidates that should have been reared for 12 months

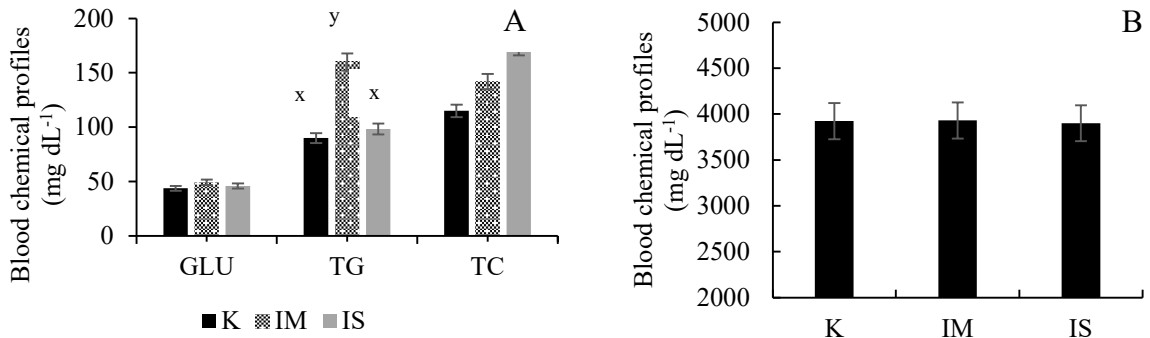
235 (Aryani & Suharman, 2015). In this study, the broodstock candidates were only 6-8 months old
 236 and reared for only 30 days.

237

238 **Blood Chemical Profiles**

1

239 Blood chemical profiles in this study include plasma cholesterol levels (total
 240 cholesterol/TC and high-density lipoprotein/HDL), plasma triglycerides (TG), and plasma
 241 glucose (GLU), which are presented in mg/dL. Blood chemical profiles of male Asian redbtail
 242 catfish broodstock candidates on pre-gonadal maturation are depicted in Figure 1.



243
 244

245 Figure 1. Blood chemical profiles (GLU, TG, TC = A; HDL = B) of male Asian redbtail catfish
 246 broodstock candidates fed with oil-supplemented diets. All values are presented in
 247 average ± SD. Different letters (x,y) above the bar indicate significantly different
 248 values ($p < 0.05$). K = Supplementation of oil combination absence (0%/control); IM =
 249 Dietary supplementation of fish oil (2%) and maggot oil (2%); IS = Dietary
 250 supplementation of fish oil (2%) and palm oil (2%). GLU = Plasma glucose (mg dL⁻¹);
 251 TG = Triglycerides (mg dL⁻¹); TC = Total cholesterol (mg dL⁻¹); HDL = High-
 252 density lipoprotein (mg dL⁻¹).

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45

253 Blood chemical profiles of male Asian redbtail catfish broodstock candidates showed no
 254 significant difference in all parameters ($p > 0.05$), except the triglyceride level (TG). The dietary
 255 combination of fish oil (2%) and maggot oil (2%) yielded the highest TG level ($p < 0.05$), yet
 256 showed no significant differences among the other treatments in GLU, HDL, and TC levels
 257 ($p > 0.05$). This condition indicates that fish fed with fish and maggot oil-supplemented diet
 258 offer a higher energy supply for steroidogenesis and testicular membrane formation (Kumar et
 259 al., 2018), specifically during pre-gonadal maturation and deposition, than other treatments.

260 Steroidogenesis occurs when cholesterol, including triglycerides, is converted into steroid
261 hormones, like testosterone and progesterone (17α , 20β -dihydroxy-4-pregnen-3-one/DHP)
262 (Soranganba & Singh, 2019), while maintaining the aggressiveness level from the male fish
263 that may coexist with chemical signalling and social cues in rearing media (Saraiva *et al.*,
264 2017). Similarly, Egessa *et al.* (2025) reported that dietary supplementation with fish oil and
265 maggot oil (ratio 1:1) did not result in a significant difference in total cholesterol levels in
266 African catfish hybrids (*Clarias gariepinus* \times *Heterobranchus longifilis*), except for glucose
267 levels. Moreover, He *et al.* (2024) reported that a dietary combination of fish and maggot oil
268 (25-75%) showed no significant differences in total cholesterol and triglyceride levels in white
269 shrimp (*Litopenaeus vannamei*), except for HDL levels.

270 Although no significant differences in other blood profile parameters were observed,
271 the following condition is reasonable, as triglycerides (TG) are more sensitive to changes in
272 dietary lipid supply than HDL and CHO levels (Mohamed *et al.*, 2019). As also reported by
273 Shen *et al.* (2022) in juvenile black seabream *Acanthopagrus schlegelii* after being fed with
274 fish oil, palm oil, and soybean oil at high concentration level in diets (13.5%) and Gong *et al.*
275 (2024) in largemouth bass (*Micropterus salmoides*) fed with 7% soybean oil-supplemented
276 diet, unchanged HDL, CHO, and GLU may indicate an optimal condition during pre-gonadal
277 maturation, where broodstock candidates were more prone to perform reproduction process,
278 that required more TG level, while still regulating optimal nutrient transport and nutrient
279 deposition, as parts of body maintenance components. In addition, according to Sattang *et al.*
280 (2021), maggot oil contains saturated fatty acid (lauric acid C12:0) and polyunsaturated fatty
281 acids (PUFA), like arachidonic acid (20:4n-6), that may be associated with high TG level in
282 male broodstock candidates for further gonadal development, namely spermatogenesis.
283 Therefore, for further optimal application, a dietary combination of fish oil (2%) and maggot

284 oil (2%) ensures the lipid deposit improvement and energy supply for further gonadal
285 maturation in male Asian redbtail catfish broodstock candidates.

286

287 **CONCLUSIONS**

288 This study concludes that dietary supplementation of animal-based and plant-based oils
289 had no effect on pre-gonadal maturation of male Asian redbtail catfish. However, in blood
290 chemical profiles, the highest triglyceride level found in animal-based oil dietary
291 supplementation (fish oil and maggot oil) may provide a ready-to-use energy for further
292 gonadal maturation in male Asian redbtail catfish broodstock candidates, before the broodstock
293 selection program begins.

294

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309 AUTHOR CONTRIBUTION

43 310 TM: Conceptualization, formal analysis, supervision and writing – review and editing;
38 311 SSQT: Conceptualization, methodology, and writing – original draft preparation; MA:
13 312 Methodology, formal analysis, and visualization; SJR, MASO, RTA, and AF: Investigation, project
313 administration, and writing – original draft preparation.

314

9 315 DECLARATION OF COMPETING INTEREST AND USE OF GENERATIVE AI

32 316 The authors declare that there are no competing interests. During the preparation of this
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