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## A meta-analysis of integrated multi-trophic aquaculture

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1 **A meta-analysis of integrated multi-trophic aquaculture: Extractive species growth is**  
2 **most successful within close proximity to open-water fish farms**

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10  
11 **Running title**

12 Extractive species in open-water IMTA

13  
14 **Abstract**

15  
16 Fish farming in open water releases dissolved and particulate waste (inorganic and organic)  
17 into the surrounding marine environment. To reduce this environmental impact, commercial  
18 extractive species can be grown alongside to utilise and reduce this waste, a technique known  
19 as integrated multi-tropic aquaculture (IMTA). Information is lacking on whether: 1) IMTA is  
20 generally successful with respect to extractive species growth responses; 2) at what spatial  
21 scale they can be cultivated from fish cage nutrient sources. Focussing on bivalves and  
22 macroalgae as extractive species, this study uses a meta-analysis approach to summarise and  
23 conclude peer reviewed data on IMTA to address these information gaps. We show that there  
24 are clear benefits to integrating bivalves and macroalgae with fish farms. Bivalves grown  
25 within, and relatively near, fish cages (0 m and 1-60 m distance categories respectively)  
26 showed significantly higher biomass production relative to controls compared to those grown  
27 at larger spatial scales (61+ m). However, biomass production of macroalgae was  
28 significantly higher than controls only within close proximity to fish cages (0 m). This  
29 information shows increased extractive species production is generally greatest at relatively  
30 small spatial scales. It also highlights the need for more site specific information (e.g.  
31 seawater parameters, hydrodynamics, food supply, farm capacity) in future studies. The  
32 allocation of control sites and locating these at suitable distances (>1 km) from fish farm  
33 effluent sources to avoid fish farm nutrient contamination is also recommended.

35 **Keywords:** Bivalves; extractive species; fish farm; integrated multi-trophic aquaculture  
36 (IMTA); macroalgae; sustainable aquaculture.

37

## 38 **1. Introduction**

39

40 Large-scale increases in the intensive mariculture of high-value carnivorous  
41 organisms can often result in a number of environmental and sustainability problems (Naylor  
42 et al, 2000; Valiela et al, 2001; Naylor et al, 2005; Cabello, 2006; Bergqvist & Gunnarsson,  
43 2013). Consequently, this has contributed towards a generally negative public perception of  
44 aquaculture, particularly in Western nations (Mazur & Curtis, 2008) which could restrict the  
45 potential for future growth in this much needed sector (White et al, 2004). A particular  
46 concern is the release of allochthonous nutrients into the surrounding water column from the  
47 rearing of carnivorous fish (e.g. Salmonids) in floating sea cages (open-water farming).  
48 Nutrients are released as both particulate (organic) waste (uneaten feed pellets and fish  
49 faeces) and as dissolved (inorganic) nutrients as a result of nutrient leaching from particulate  
50 waste and fish excretory products (Olsen & Olsen, 2008; Wang et al, 2012). Nutrient loading  
51 due to fish farming is considerable (Wang et al, 2012) and can negatively impact the benthic  
52 environment due to smothering and increased organic enrichment, leading to alterations in  
53 sediment chemistry with knock-on effects on benthic biodiversity (Giles, 2008; Olsen &  
54 Olsen, 2008; Hargrave, 2010). Many attempts to reduce nutrient loading surrounding fish  
55 farms have been made (e.g. improving the digestibility of fish feeds, computerized feed-  
56 management systems), however such technological improvements have not yet eliminated the  
57 problem of nutrient pollution associated with fish farming (Islam, 2005; Wang et al, 2012).

58 One solution to reducing the environmental impact of fish farming is the use of  
59 integrated multi-trophic aquaculture (IMTA). IMTA can be used to potentially recycle these  
60 nutrients by cultivating additional commercially relevant organisms. These ‘extractive  
61 species’ are able to intercept and assimilate aquaculture derived waste (both organic and  
62 inorganic) when cultivated alongside fed fish species (Edwards et al, 1988; Chopin et al,  
63 2001; Neori et al, 2004; Troell et al, 2009). This IMTA approach could therefore potentially  
64 bio-mitigate the negative environmental impacts of aquaculture whilst simultaneously  
65 providing a secondary marketable product for the farmer with possible economical benefit  
66 and improved public perception (Chopin et al, 2001; Troell et al, 2003; Ridler et al, 2007). In  
67 practice IMTA can take the form of a large variety of systems particularly in Asia (e.g.  
68 temporal integration of rice and shrimp or the polyculture of shrimp, fish and crabs in

69 brackish ponds; Troell, 2009) however the majority of Western IMTA operations are land-  
70 based, recirculating systems successfully rearing crops of finfish, macroalgae and  
71 macroalgivores (e.g. Neori et al, 1996). The majority of general Western aquaculture  
72 activities are carried out at sea but at present, there are relatively few commercial examples of  
73 open-water IMTA systems (Barrington et al, 2009).

74 A variety of organisms (e.g. echinoderms or crustaceans; Cook & Kelly, 2007;  
75 Barrington et al., 2009; Nelson et al, 2012) have been included as part of open-water IMTA  
76 trials, however the most commonly cultivated extractive groups are bivalves and macroalgae.  
77 In contrast to echinoderms, bivalves and macroalgae are cultivated down current of fish  
78 cages, allowing natural water currents to move farm nutrient waste towards these suspended  
79 extractive species. The groups can be divided into organic (bivalves) and inorganic  
80 (macroalgae) extractive species based on whether the group in question utilises the organic or  
81 inorganic nutrients released from fish farms. Suspension feeding bivalves are generalist  
82 consumers, able to ingest a variety of particle types and sizes, therefore particulate fish waste  
83 could provide an additional food source for bivalves (Jones & Iwama, 1991; Troell et al,  
84 2003). Laboratory and field studies utilizing stable isotopes and fatty acids as biomarkers  
85 have confirmed that bivalves (*Mytilus edulis*, *Mytilus galloprovincialis*, *Perna viridis*) are  
86 able to capture and assimilate fish farm derived organic waste (Lefebvre et al, 2000; Mazzola  
87 & Sara, 2001; Gao et al, 2006; Reid et al, 2010; Redmond et al, 2010; MacDonald et al,  
88 2011). Similarly, Pacific oysters (*Crassostrea gigas*) have demonstrated high growth rates  
89 ( $0.7\% \text{ day}^{-1}$ ) when used as biofilters in land-based IMTA systems (Shpigel, 2005). Although  
90 mathematical models have suggested that the capacity of bivalves to assimilate farm derived  
91 waste may be limited in an open-water context (Cranford et al, 2013), the high food supply  
92 environment surrounding fish farms potentially provides an opportunity for increased bivalve  
93 growth (Page & Hubbard, 1987; Brown & Hartwick, 1988).

94 Macroalgal species chosen for inclusion within open-water IMTA operations are  
95 typically those with value either as a foodstuff (e.g. *Saccharina latissima*), for industrial  
96 applications such as agar extraction (e.g. *Gracilaria spp*), or the cosmetics market (e.g.  
97 beauty spas and products). Most are capable of utilising ammonium cations ( $\text{NH}_4^+$ ) which is  
98 the primary nitrogen species emitted by fish farms (Hanisak, 1983; Chen et al, 2003;  
99 Fernandez-Jover et al, 2007). Nitrogen availability is often a major constraint limiting  
100 macroalgal growth, particularly in temperate but also some tropical regions (Lobban &  
101 Harrison, 1996; Larned, 1998). Therefore, in areas where macroalgal growth is nitrogen  
102 limited (e.g. northern temperate regions) greater availability of inorganic nitrogen found

103 within the vicinity of fish farms could result in increased macroalgal growth rates (Chopin et  
104 al, 2001; Neori et al, 2004). Ammonium levels immediately surrounding fish farms have been  
105 observed to be below the saturation threshold for macroalgae such as *S. latissima* (Ahn et al,  
106 1998) and *Gracilaria vermiculophylla* (Abreu et al, 2011a) suggesting that macroalgae are  
107 capable of fully exploiting these available nutrients (Sanderson et al, 2012; Handå et al,  
108 2013). Such uptake has been evidenced within controlled land-based IMTA systems, with 72  
109 % of nitrogen removed concurrent with increased macroalgal growth (e.g. Neori et al, 2000;  
110 Chopin et al, 2001; Matos et al, 2006; Abreu et al, 2011b). Based on this evidence, it can be  
111 expected that macroalgal species will show high growth rates in the vicinity of fish farm  
112 structures releasing high inorganic nutrient loads.

113 Land-based IMTA systems are mostly closed loop systems thus allowing control of  
114 nutrient rich waste (Chopin et al, 2001). In contrast, open-water IMTA lacks this fine control  
115 with the dilution of waste occurring by natural seawater movement (e.g. currents). These  
116 systems are however generally sheltered within fjordic systems (e.g. Scottish sea lochs) and  
117 are likely to have regular current patterns (Navas et al, 2011) leading to the general  
118 assumption that the organic and inorganic nutrients will progressively disperse as distance  
119 increases from the farm. This increased dilution with distance from farm effect may severely  
120 affect the ability of extractive species to intercept nutrient rich waste, thus raising concerns  
121 over the effectiveness of using IMTA within an open-water context (Cranford et al, 2013).  
122 There is therefore a knowledge gap on the spatial scale at which extractive species can be  
123 located in order to assimilate waste and increase profitability.

124 This study will focus only on extractive species growth at different spatial scales to  
125 determine whether IMTA can be regarded as worthwhile. Individual trials investigating the  
126 cultivation of macroalgae as an extractive species show some indication of positive results  
127 whereas those using bivalves as extractive species are less clear. To date this information has  
128 not been collated in an informative manner. An overview to determine the effectiveness of  
129 open-water IMTA in the context of the production of extractive species is therefore required.  
130 Such an overview could help stakeholders determine whether IMTA practices are worth  
131 adopting, as well as contributing towards Blue growth (Whitmarsh et al, 2006; DEFRA,  
132 2015).

133 This study aims to summarise from the available literature whether open-water IMTA  
134 results in extractive species growth augmentation. More specifically, it will focus on the  
135 growth of bivalves and macroalgae cultivated in the vicinity of open-water fish farms.  
136 Growth responses at increasing distances from the fish cage will also be investigated to help

137 determine the best location to place the extractive species in relation to the farm, information  
138 which could be useful for IMTA implementation. We hypothesize that extractive species will  
139 show increased growth (relative to controls) when cultured alongside fish farms with growth  
140 augmentation declining as distance increases from the closest fish cage.

141

## 142 **2. Methods**

143

### 144 *2.1. Data selection*

145

146 A comprehensive search of peer reviewed literature was carried out during early 2015  
147 using a keyword search of the Web of Science database. Studies were located using the  
148 terms; “bioremediation”, “bivalve”, “growth”, “IMTA”, “integrated aquaculture”,  
149 “macroalgae”, “mussel”, “polyculture”, “salmon” and “seaweed” with those studies of direct  
150 relevance to the subject matter of this study selected for use. All literature selected compared  
151 the growth rate of extractive species (bivalves or macroalgae) cultivated in the vicinity of  
152 commercial open-water fish farms with an expressly specified control. Studies which did not  
153 include a designated control were excluded from this analysis. All studies were experimental  
154 interventions except for Wallace (1980) who measured the growth of naturally occurring  
155 fouling mussels on artificial structures both in the vicinity and at a distance from fish cages.  
156 Only studies which provided all necessary data (growth parameters, standard deviation  
157 values, sample size) were included within this analysis. Data were restricted to the use of  
158 cultivated extractive species of potential commercial interest (Bivalves: *Crassostrea gigas*,  
159 *Mytilus edulis*, *Mytilus galloprovincialis*, *Mytilus planulatus*, *Ostrea edulis* and *Placopecten*  
160 *magellanicus*; Macroalgae: *Gracilaria chilensis*, *Palmaria palmata*, *Sargassum hemiphyllum*,  
161 *Sargassum henslowianum*, *Saccharina latissima* and *Ulva spp*). Data were further restricted  
162 to those studies which quantified shell length for bivalves and the parameters blade length  
163 (cm), biomass production (kg fresh mass m<sup>-1</sup>) and specific growth rate (SGR; % day<sup>-1</sup>) for  
164 macroalgae. The latter was averaged across the total length of time for each respective study.  
165 Data were extracted from graphical figures within the literature using digitizing software  
166 (PlotDigitizer; <http://plotdigitizer.sourceforge.net>).

167 Several of the studies included within the meta-analysis contributed more data points  
168 than other studies. For example, Navarrete-Mier et al (2010) measured the growth of two  
169 extractive species (*O. edulis* and *M. edulis*) at five different distances (0 m, 25 m, 120 m, 300  
170 m & 600 m) from the nearest fish farm thereby contributing ten data points to the meta-

171 analysis. In contrast, measuring the growth of one extractive species (*C. gigas*) at one  
172 distance (e.g. Jiang et al, 2013) contributed only a single data point towards the meta-  
173 analysis. In this study the difference in growth in extractive species between experimental  
174 (IMTA) and control sites for each distance was treated as a separate data point, providing the  
175 selection criteria described above were met. Although using multiple observations from a  
176 single study can decrease the independence of these data points, it was necessary due to the  
177 limited number of studies suitable for inclusion (e.g. Kroker et al, 2010).

178

## 179 2.2. Data analysis

180

181 All studies used in this meta-analysis compared the growth rate of extractive species  
182 grown in the vicinity of open-water fish farms with those of a designated control, therefore  
183 standardized mean difference was used as the effect size. Effect size is used to quantify the  
184 magnitude of difference between two groups with the difference expressed in standard  
185 deviation units (Sullivan & Feinn, 2012). A positive value for effect size indicates the  
186 experimental group outperformed the control group, a negative effect size indicates  
187 underperformance. An effect size of zero indicates no difference between experimental and  
188 control groups. For each data point, standardized mean difference was expressed as Cohens  
189  $d'$  which was calculated using Formula 1 (Gurevitch et al, 2001; Lakens, 2013) where  $M^E$  and  
190  $M^C$  represent mean extractive species size at the end of the experimental period for the  
191 experimental and control groups respectively. The use of Cohens  $d'$  can give a biased  
192 estimate of effect size when sample sizes are small ( $< 20$ ) or differ between experimental and  
193 control groups (Hedges & Olkin, 1985). This was encountered within this analysis, therefore  
194 an unbiased corrected effect size (Hedges  $g'$ ) was used in this analysis. Hedges  $g'$  was  
195 calculated from Cohens  $d'$  using Formula 2 where  $n_E$  &  $n_C$  represent sample size for the  
196 experimental and control groups respectively (Gurevitch et al, 2001; Lakens, 2013). Variance  
197 in effect size ( $V_D$ ) can be calculated by squaring standard error in effect size ( $SE_D$ ) which is  
198 calculated using Formula 3 (Gurevitch et al, 2001; Lakens, 2013), where  $d$  is effect size  
199 (Hedges  $g'$ ).

200

201 **Formula 1:** Cohens  $d = (M^E - M^C) / SD_{pooled}$

202 **Formula 2:** Hedges  $g' = Cohens\ d' \times [1 - (3/4)(n_E + n_C - 9)]$

203 **Formula 3:**  $SE_D = \sqrt{[(n_E + n_C) / n_E n_C] + [d^2 / 2(n_E + n_C)]}$

204

205 The studies used in this analysis varied due to interspecific differences between  
206 species (e.g. growth rate) and variation in site-specific conditions (e.g. temperature, salinity  
207 & chlorophyll-*a* levels). Therefore, a random-effects model was used to calculate the  
208 weighted mean effect size. Random-effects models account for two sources of sampling  
209 error; within-study variance and between-study variance. Within study variance is given by  
210  $V_D$  (see above). Between study variance ( $r$ ) was calculated by subtracting the degrees of  
211 freedom ( $n-1$ ) from total variance and then dividing by a scaling factor, using equations given  
212 by Borenstein et al, 2007. Total variance ( $V_D^*$ ) for each data point was calculated by adding  
213 together  $V_D$  and  $r$ . The reciprocal of  $V_D^*$ ,  $w_i$  was used to determine the weighting each data  
214 point carried within the combined effect.

215 For each study a weighted mean effect size ( $T$ ) was calculated. All data points from  
216 each study were combined using Formula 4 (Borenstein et al, 2007) where  $T_i$  is effect size  
217 (Hedges  $g'$ ). The standard error of mean effect size ( $SE_T$ ) was calculated using Formula 5.  
218 The significance of weighted mean effect size was assessed by constructing 95%  
219 bootstrapped confidence intervals around weighted mean effect size using equations given by  
220 Borenstein et al (2007). If 95% confidence intervals do not cross zero, weighted mean effect  
221 size can be considered significant (Borenstein et al, 2007). Forest plots were constructed to  
222 show the results graphically (weighted mean effect size  $\pm$  95% confidence intervals). Studies  
223 which contributed a single data point (e.g. Sara et al, 2009) were presented simply as Hedges  
224  $g'$  for graphical representation of effect size. The total weighted mean effect size ( $T^*$ ) was  
225 then calculated by combining all of these weighted data points from all studies for bivalves  
226 and macroalgae (Formula's 4 & 5). All calculations were performed on Microsoft Excel 2016  
227 (version 16.0) using the framework provided by Neyeloff et al (2012) as a guide.

228

229 **Formula 4:**  $T = \Sigma (w_i T_i) / \Sigma w_i$

230 **Formula 5:**  $SE_T = \sqrt{(1/w_i)}$

231

### 232 *2.3 Distance subgroup analysis*

233

234 To determine the effect of distance on the growth of extractive species, each data  
235 point was categorized into a subgroup. Bivalve data points were categorized into four  
236 distance categories between the bivalves and the nearest stocked fish cage; 1) 0 m, 2) 1-60 m,  
237 3) 61-299 m and 4) 300+ m. "0 m" indicates the bivalves were located inside, suspended  
238 underneath or "immediately adjacent" to the fish cage. Previous studies have reported that 99



239 % of particles originating from fish farms will settle within 60 m (Coyne et al, 1994; Giles,  
240 2008), therefore a distance category of “1-60 m” was also used in this analysis. Not all  
241 studies reported an explicit distance between bivalves and fish cages, studies which stated  
242 experimental bivalves were located “adjacent” to fish cages were presumed to be between 1  
243 and 60 m of fish cages and thus were included within this distance category. Bivalves taken  
244 from floats supporting fish farms (Wallace, 1980) were also included within the “1-60 m”  
245 category. To maximise categorical balance, the threshold between the 3<sup>rd</sup> and 4<sup>th</sup> distance  
246 categories was set at an arbitrary value of 300 m.

247 Macroalgae data points were categorized into three distance categories; 1) 0 m, 2) 1-  
248 60 m and 3) 61+ m. “0 m” indicates macroalgae were cultivated “within” or “attached” to the  
249 fish farm. Major nutrient enhancement is found within 60 m of fish farms (Sanderson et al,  
250 2008), therefore, similar to bivalves (described above) the next category was set as “1-60 m”.  
251 Not all studies reported an explicit distance between macroalgae and fish cages. Studies  
252 which stated that macroalgae were located “adjacent” to fish cages were presumed to be  
253 between 1 and 60 m of fish cages and thus were included within this distance category. As  
254 only five data points were made at distances exceeding 60 m, to maximise categorical  
255 balance the final category was therefore set as “61+ m”. A weighted mean effect size with  
256 95% confidence intervals was then constructed for each individual study and subgroup in  
257 addition to total weighted mean effect size using the methods described above.

258

#### 259 *2.4. Sensitivity*

260

261 To test the robustness of our findings, a sensitivity analysis was performed using the  
262 method employed by Kroeker et al (2010). To summarise, those studies with the largest effect  
263 size (regardless of distance) were systematically removed from the meta-analysis, which was  
264 then re-run to determine what effect removal had on the meta-analysis outcome. This step  
265 was then repeated with effect sizes of decreasing magnitude to determine how many studies  
266 needed to be removed to change the significance of the overall result. Similarly, if any study  
267 contributed five or more data points to the meta-analysis then this study was removed and the  
268 meta-analysis re-ran.

269

### 270 **3. Results**

271

#### 272 *3.1. Bivalves*

273

274 Twelve studies were found which compared the growth of bivalves cultivated in the  
275 vicinity of fish farms with designated controls. From these 12 studies, 43 data points were  
276 extracted and incorporated within the meta-analysis. The analysis showed that IMTA had an  
277 overall significantly positive effect on the growth rate of bivalves, as indicated by total  
278 weighted mean effect size ( $T^*$ ; Figure. 1). However, the growth augmentation bivalves  
279 experienced in open-water ITMA systems varied according to distance from the closest fish  
280 farm. Bivalves within the 0 m and 1-60 m subgroups showed significantly higher growth than  
281 controls whereas bivalves grown at further distance points (61 – 299 m and 300+ m) grew at  
282 a similar rate to control bivalves (Figure. 2).

283 The stepwise removal of the fifteen largest effect sizes and the removal of the three  
284 studies which contributed five or more data points (Jones & Iwama, 1995; Navarrete-Mier et  
285 al, 2010; Lander et al, 2012) did not alter the significance of either total weighted mean effect  
286 size or weighted mean effect size for each distance category. The sensitivity analysis  
287 therefore indicates that the findings of this meta-analysis are robust.

288

### 289 3.2. *Macroalgae*

290

291 Eight studies were found which compared the growth of macroalgae cultivated in the  
292 vicinity of fish farms with designated controls. From these eight studies, 24 data points were  
293 extracted and incorporated within the meta-analysis. The analysis showed that IMTA had an  
294 overall significantly positive effect on the growth of macroalgae, as indicated by total  
295 weighted mean effect size ( $T^*$ ; Figure. 3). However, the growth augmentation macroalgae  
296 experienced in open-water ITMA systems varied according to distance from the closest fish  
297 farm (Figure. 4). Macroalgae within the 0 m subgroup grew significantly faster than controls  
298 whereas macroalgae grown further in distance (1-60 m and 61+ m) grew at a similar rate to  
299 control macroalgae.

300 A single study (Sanderson et al, 2012) contributed five or more data points to this  
301 meta-analysis. The removal of Sanderson et al (2012) did not alter the significance of total  
302 weighted mean effect size. However, the removal of the five largest effect sizes from the  
303 database used to calculate total weighted mean effect size altered the significance of total  
304 weighted mean effect size (significant to non-significant). Stepwise removal of high  
305 magnitude data points from the subgroup analysis did not alter the significance of any  
306 findings. As the removal of high magnitude data points altered the significance of total

307 weighted mean effect size, the findings for macroalgae are less robust than for bivalves.

308

#### 309 **4. Discussion**

310

311 The growth of extractive species (bivalves and macroalgae) was significantly greater  
312 than controls when integrated with open-water fish farms. Macroalgae cultivated within fish  
313 farms (0 m) performed significantly better than those at increasing distances. Bivalves  
314 cultivated within (0 m) and near (1-60 m) fish farms showed significantly greater growth than  
315 those located at further distances (61+ m). Extractive species therefore show best growth  
316 performances when located within close proximity to fish farms. Macroalgae generated higher  
317 growth rates when integrated within the farm but bivalves showed a larger spatial scale of up to  
318 60 m distance for highest growth performances. Overall, these results demonstrate that IMTA  
319 is effective with respect to extractive species augmentation which could help farmers generate  
320 a profit, particularly if compared to monospecific farms. Although these results lend support to  
321 the implementation of open-water IMTA systems, the meta-analyses showed high variation.  
322 Such variation can be attributed to many factors which we will now discuss.

323

##### 324 *4.1. Sources of variation*

325

###### 326 *4.1.1. Seasonality and nutrient / food supply*

327

328 The growth enhancement of macroalgae cultivated as part of open-water IMTA  
329 systems can be attributed to a fertilisation effect due to increased nutrient levels found within  
330 fish farms. Further evidence for the utilisation of farm derived inorganic nutrients is provided  
331 by the increased nitrogen content of macroalgae cultivated 10 m from fish cages and by the  
332 enrichment of *S. latissima* in a nitrogen isotope ( $\delta^{15}$ ) typical of fish effluent (Troell et al, 1997;  
333 Sanderson et al, 2012). Many factors can influence nutrient levels surrounding fish farms  
334 including feeding regime, hydrodynamics (Sanderson et al, 2008) and ambient nutrient levels.  
335 Macroalgal growth is often limited during the summer due to low ambient nutrient levels  
336 (Lobban & Harrison, 1996), it can therefore be expected that IMTA macroalgae will  
337 experience the greatest growth enhancement during summer months due to the availability of  
338 dissolved nutrients released by nearby fish farms (Chopin et al, 2001; Neori et al, 2004).  
339 Studies included within this meta-analysis support this theory with Abreu et al (2009)  
340 observing greatest growth rates of *G. chilensis* during the summer. Similarly, Handå et al

341 (2013) found the growth enhancement of IMTA macroalgae to be most pronounced in summer  
342 and Wang et al (2014) observed the growth increase of *S. latissima* to occur at a time when  
343 dissolved nitrogen levels were at their lowest. In contrast, Halling et al (2005) found no  
344 significant difference in macroalgal biomass production between IMTA and control sites.  
345 Although ambient nutrient levels were not expressively measured by Halling et al (2005), the  
346 findings of this study may have been influenced by seasonality given that the study occurred  
347 through the austral winter (when ambient nutrients may not limit macroalgal growth). Troell et  
348 al (1997) who cultivated the same species (*G. chilensis*) at the same site but instead during  
349 summer months reported a 40% increase in integrated macroalgae production when compared  
350 to controls. These examples provide strong evidence that the seasonal timing of IMTA trials  
351 may influence the results. It is therefore likely that macroalgae cultivated by Halling et al  
352 (2005) may not have received the full benefits of integration due to their selected growth  
353 period.

354 Other seasonality related factors may also influence the growth benefit macroalgae  
355 experience when integrated with open-water fish farms. Nutrient emissions from open-water  
356 fish farms vary, but generally increase during the course of the grow-out cycle (typically two  
357 years) peaking (up to four-fold) in late summer of the second year when fish feeding levels are  
358 highest (Strain & Hargrave, 2005; Reid et al, 2013a). Commercial macroalgal harvest would  
359 have to occur during early to mid-summer as macroalgae begins to degenerate in late summer  
360 (Lobban & Harrison, 1996), therefore peak nutrient emissions from fish farms would not be  
361 available to IMTA extractive macroalgae. Consequently, for a large period of the year  
362 (particularly during the first year of fish rearing) macroalgae would not be exposed to  
363 substantially elevated nutrient levels. How macroalgal growth augmentation varies during the  
364 typical two year grow-out cycle has yet to be assessed. Such information is required because  
365 commercial IMTA ventures which integrate macroalgae will need to account for variations in  
366 farm derived nutrient emissions e.g. by scaling back macroalgal cultivation during the first  
367 year of fish growth.

368 Food availability for bivalves varies due to a variety of factors (e.g. light, nutrient  
369 levels) that undergo regular spatial and temporal fluctuations (Page & Hubbard, 1987; Navarro  
370 & Thompson, 1995; Litchman, 1998; Cranford & Hill, 1999). At mid to high latitudes  
371 phytoplankton levels are at their seasonal minima throughout the winter, therefore natural  
372 populations of bivalves often show minimal growth during this period of food limitation  
373 (Malouf & Breese, 1977; Hilbish, 1986). Wallace (1980) suggested that the faster growth rates  
374 of *M. edulis* observed at fish farms was likely due to mussels receiving a continuous supply of

375 farm derived waste throughout the winter, thus facilitating year round growth. Similar  
376 conclusions were made by Lander et al (2012) who found that the growth advantage gained by  
377 *M. edulis* in the vicinity of fish farms was most pronounced in the autumn and winter months.  
378 The importance of seasonal timing could explain why some studies found bivalves showed no  
379 significant growth enhancement through IMTA. Part of the study carried out by Cheshuk et al  
380 (2003) was conducted on an empty farm (no fish present) during the austral winter period (3 ½  
381 months from June to September). During this time these mussels (*M. planulatus*) were not  
382 exposed to farm derived waste. Chlorophyll-*a* measurements showed that natural food  
383 availability was at its lowest during this time, thus leading to the low growth rates reported.

384 When ambient particle concentration, often described as total particulate matter  
385 (TPM), exceed a certain threshold (e.g. 5.0 mg TPM l<sup>-1</sup> for *M. edulis*) then a significant  
386 proportion of ingested particles are not digested but instead are rejected as pseudofaeces  
387 (Widdows et al, 1979). Saturation of mussel feeding due to high ambient TPM was suggested  
388 by Cheshuk et al (2003) as a possible mechanism for why only modest enhancement of *M.*  
389 *planulatus* growth was observed. The use of bivalves as extractive species in open-water IMTA  
390 fish farms is dependent on bivalves directly consuming particulate fish waste. The validity of  
391 such systems may therefore be compromised if ambient particle concentrations surrounding  
392 fish farms were consistently higher than the pseudofaeces threshold. Therefore, bivalve growth  
393 enhancement may likely be achieved in IMTA systems located in areas with seasonally or  
394 consistently low ambient seston levels (Troell & Norberg, 1998). In oligotrophic waters, farm  
395 derived nutrients could also stimulate local phytoplankton production thereby increasing the  
396 food supply for secondary consumers e.g. bivalves (Sara et al, 2009). The relationship between  
397 bivalve-fish IMTA and variations in local food supply is complex and will require more focus  
398 in future IMTA studies. Quantification of the assimilation of farm derived waste at varying  
399 particle concentrations would assist in elucidating the relationship between the outcome of  
400 open-water IMTA and ambient seston levels.

401

#### 402 4.1.2. Hydrodynamics

403

404 Models indicate that bivalves are best able to capture particulate fish waste when  
405 cultivated in areas with slow (< 0.05 m s<sup>-1</sup>) current speeds (Troell & Norberg, 1998; Cranford  
406 et al, 2013). Studies included within this meta-analysis measuring faster current speeds of up to  
407 0.11 m s<sup>-1</sup> (e.g. Navarette-Mier et al, 2010) found no evidence for bivalve growth  
408 augmentation. Particle capture efficiency is dependent on the amount of time available to filter

409 particles from the surrounding water column which is dependent on current speed (fast current  
410 speeds equal less time to extract food particles and *vice versa*). IMTA bivalves cultivated in  
411 areas where currents do not regularly exceed  $0.05 \text{ m s}^{-1}$  can therefore be expected to show  
412 greater growth enhancement when integrated with open-water fish farms (Troell & Norberg,  
413 1998; Cranford et al, 2013).

414 Nutrient dispersal surrounding open-water fish farms is influenced by hydrodynamics,  
415 subsurface geographical features and the structure of the fish cage (Sanderson et al, 2008).  
416 Understanding dispersal patterns and how they change over time is a complex task requiring  
417 extensive field work and advanced modelling (Olsen et al, 2008). An understanding of nutrient  
418 emissions from open-water fish farms (also referred to as volumetric loading) would be of  
419 importance for commercial IMTA ventures, because it would allow farmers to obtain the  
420 maximum growth benefit for their crop through optimum placement of extractive species.  
421 Optimum placement is likely to be highly site-specific therefore this meta-analysis cannot  
422 provide detailed information on how to organise a commercial IMTA farm besides showing the  
423 general distances at which extractive species can be cultivated.

424

#### 425 *4.1.3. Species specific responses*

426

427 Species specific differences in fish faecal properties, extractive species optimal  
428 growth, assimilation and feeding mechanisms and patterns will have contributed towards our  
429 results. Bivalve growth rates differ intrinsically between species, as shown by comparative  
430 studies (Epifanio, 1979; Laing et al, 1987; Cardoso et al, 2006). Intra-specific variation could  
431 thus have contributed to the varying growth responses seen in IMTA bivalves as seen by  
432 Rensel et al (2011). However, given the small number of studies suitable for inclusion in this  
433 meta-analysis, the data were not used to determine the species specific level of contribution, of  
434 farmed fish or extractive species, to the effect sizes reported in this study. More open-water  
435 IMTA intervention studies on a range of farmed fish and extractive species types would help to  
436 determine which species provide the biggest influence on IMTA responses. Despite this  
437 variation, increased extractive species growth found during this study demonstrates that  
438 increased extractive species production is generally achievable across a range of species.

439

440

#### 441 *4.1.4. Control site selection*

442

443 Macroalgae have demonstrated increased production at distances as great as 800 m  
444 from fish farm effluent sources (Abreu et al, 2009). In some cases, control sites have been  
445 located within this range and demonstrate pronounced biomass production (Halling et al,  
446 2005). It is therefore likely that these sites have been located within the dispersal range of fish  
447 farm effluents and therefore could mask potential farm specific responses. To avoid  
448 downstream nutrient contamination of controls in future studies, we recommend that control  
449 site location considers the hydrodynamics of the area and suitably high distances (e.g. > 1 km  
450 (8 km as used by Abreu et al (2009)) from fish cages. Furthermore, the selection criteria for  
451 this meta-analysis outlined that literature which did not include a designated control were  
452 excluded from the analysis, and as a consequence several studies were not used in the analysis.  
453 We therefore recommend the use of well-placed controls in future intervention experiments to  
454 help increase the body of evidence around IMTA responses.

455

#### 456 *4.1.5. Site specific information*

457

458 The description of site specific conditions was variable or sometimes absent in the  
459 literature (e.g. chlorophyll-*a* concentration, mean current speed). This information is required  
460 to understand what effects (if any) site-specific conditions have on the capacity of extractive  
461 species to capture and assimilate fish waste. This information is also valuable to farmers by  
462 allowing identification of localities where commercial IMTA ventures are most likely to  
463 succeed. Based from our experience in this meta-analysis we recommend that future studies  
464 consider the following information for study areas: water temperature and salinity, mean and  
465 maximum current speeds and their direction, chlorophyll-*a* concentration, particulate organic  
466 matter and TPM concentrations. Additionally, details on fish-farm size and feeding protocols  
467 would be beneficial towards understanding the effect of distance between fish cages and  
468 extractive species.

469

#### 470 *4.2. Meta-analysis limitations*

471

472 One of the limitations with meta-analysis is the reliance on publicly available data  
473 which could create a bias on the reported effect sizes. This is a possibility for the data used  
474 within this meta-analysis because the majority (80 %) of the macroalgal growth responses  
475 included in this meta-analysis demonstrated strong evidence for increased macroalgal growth.  
476 Half of the bivalve studies used in this meta-analysis reported positive growth responses

477 relative to controls. It is possible that data has not been made publicly available from studies  
478 which were unsuccessful in extractive augmentation within IMTA. Such information will be  
479 critical for future meta-analytical summaries of IMTA implementation as more literature is  
480 released in this field of research. It is therefore highly recommended that researchers and  
481 journals encourage publicising data which demonstrates unsuccessful extractive  
482 augmentation within IMTA to prevent possible future bias.

483 The initial literature search identified 14 studies which cultivated macroalgae in the  
484 vicinity of open-water fish farms. However, six of these studies were excluded from  
485 subsequent analysis due to the lack of an expressly specified control or a lack of reporting of  
486 data (e.g. sample size) required for the meta-analysis. Therefore, only eight macroalgal  
487 studies (containing 24 data points) were included within this analysis, compared to 12 bivalve  
488 studies (containing 43 data points). Given that significant bivalve growth enhancement was  
489 found within the 1-60 m distance category despite the generally rapid settling velocity of  
490 particulate fish waste (Law et al, 2014), the lack of significant macroalgal growth  
491 enhancement at distances greater than 0 m could be deemed surprising. The paucity of  
492 suitable macroalgal studies could be a potential causative factor behind the lack of significant  
493 macroalgal growth enhancement at distances greater than 0 m. This therefore emphasises the  
494 recommendation for future IMTA studies to include suitable controls within experimental  
495 designs as it is only with reference to controls that the presence (or lack of) growth  
496 enhancement can be determined.

497

#### 498 *4.3. Logistics of extractive species in IMTA*

499

500 As the dilution of particulate fish waste increases with distance from fish cages  
501 (Doglioli et al, 2004), bivalves cultivated within fish cages themselves (0 m) will be exposed to  
502 higher concentrations of particulate waste (and thus increased food availability) than those at  
503 greater distances. Therefore, greater growth augmentation for bivalves and macroalgae within  
504 the 0 m subgroup is predictable. However, significantly increased bivalve growth was also  
505 observed within a larger spatial scale, in this case up to 60 m. Although significant macroalgal  
506 growth augmentation was only found for the 0 m subgroup, individual studies have found  
507 significantly increased macroalgal production at distances of up to 800 m from fish farms  
508 (Abreu et al, 2009). Such findings are encouraging as farmers are unlikely to adopt IMTA  
509 practices if the installation of extractive species interferes with the day to day operations of the  
510 fish farm (the major cash crop) e.g. restricting access to fish cages or impeding water flow



511 (thus reducing oxygen supply to fish). Therefore, extractive species must be appropriately  
512 located (e.g. not inside a fish cage). The results of this meta-analysis indicate that spatial  
513 constraints may not represent an impediment to widespread open-water IMTA. To maximise  
514 farm waste recapture (as well as biomass production), bivalves should be cultivated close to  
515 fish cages due to the rapid settling velocity of particulate fish waste (Law et al, 2014).  
516 However, care should be taken in locating the macroalgal component of open-water IMTA  
517 farms as excess particulate fish waste could potentially settle on macroalgal fronds thus  
518 blocking light and restricting growth.

519         The evidence presented in this study shows that by adopting IMTA practices,  
520 economic advantages could be gained by farmers (though increased production of extractive  
521 species). By providing secondary marketable crops, IMTA farms exhibit a greater degree of  
522 economic diversification compared to monoculture operations. Diversification represents a  
523 form of insurance, as a marketable product will still be produced in the event of disease  
524 outbreaks or infrastructure damage (e.g. net failure). Profitable markets presently exist for the  
525 sale of bivalves (Lucas & Southgate, 2012) and the farmed seaweed market is likely to grow in  
526 Western nations given the increasing popularity of seaweed consumption (Brownlee et al,  
527 2012). Much of the infrastructure and equipment for IMTA (e.g. rope lines, boats, buoys) will  
528 already be present on fish farms, therefore the start-up costs of IMTA in farms is likely to be  
529 low (Lander et al, 2012). However, labour costs can be expected to increase on IMTA farms as  
530 extra work hours will be required for the maintenance and harvesting of extractive species  
531 (Holdt & Edwards, 2014). A model based on a Canadian farm estimates that net present value  
532 (NPV) is increased by 24% when mussels and seaweed are grown alongside Atlantic salmon  
533 (Ridler et al, 2007) however more transparent models containing greater detail (e.g.  
534 proportions of extractive species) would be of use to farmers in determining the economies of  
535 integration. Surveys have shown a positive attitude towards IMTA amongst the general public  
536 indicating that consumer acceptance will not be a barrier to IMTA expansion, with 50% of  
537 participants willing to pay 10% extra for IMTA labelled products (Barrington et al, 2010).  
538 Therefore, a system of eco-labelling may allow IMTA farmers to charge a higher price for their  
539 products and thus keep IMTA farms profitable in the face of falling fish prices (Whitmarsh et  
540 al, 2006) or competition with larger firms (Ridler et al, 2007). The profitability of IMTA farms  
541 may also be improved if coastal management systems legally oblige operations to pay for the  
542 environmental cost of their activities via discharge taxes (“user pays” concept; Troell et al,  
543 2003).

544         However, before IMTA becomes more widely implemented there are a number of

545 mitigation and biosecurity issues regarding commercial IMTA that need to be satisfactorily  
546 resolved. While bivalve integration has shown generally positive growth responses in this  
547 meta-analysis, the net organic loading from bivalves (released as faeces) combined with the  
548 fish farm may still have a negative impact on the underlying benthos. It has been  
549 recommended by the Fisheries and Oceans Canada (DFO) Science Advisory Schedule (DFO,  
550 2013) to use extractive deposit feeding species (e.g. sea urchins, sea cucumbers and  
551 polychaetes) located underneath the suspended bivalve extractive species to consume these  
552 heavy organic solids (Cranford et al., 2013; DFO, 2013; Reid et al., 2013b). The  
553 implementation of adding another trophic level into IMTA will require structural  
554 considerations relating to the fish farm (e.g. oxygen supply via seawater flow and efficient  
555 connection between trophic levels; DFO, 2013).

556 Previous work has found that IMTA bivalves grown in water of sufficient depth are  
557 highly unlikely to act as reservoirs for fish pathogens such as infectious salmon anaemia virus  
558 (ISAV) or *Vibrio anguillarum* and may assist in the control of drug-resistant pathogens and  
559 parasites such as the sea louse *Lepeoptheirus salmonis* (Mortensen, 1993; Skar & Mortensen,  
560 2007; Molloy et al, 2011; Pietrak et al, 2012; Molloy et al, 2014). Haya et al (2004) similarly  
561 found that extractive species (*M. edulis* and *S. latissima*) cultured in the vicinity of a *S. salar*  
562 farm did not accumulate hazardous therapeutants or contaminants (e.g. heavy metals) above  
563 background levels. To facilitate the spread of open-water IMTA further work regarding  
564 bioaccumulation within extractive species and potential disease transfer within farms requires  
565 consideration to dispel any concerns farmers or regulatory bodies may have regarding IMTA.  
566 If commercial IMTA is to become widespread, legislation governing aquaculture operations  
567 may have to be reformed so that policy recommendations (e.g. minimum distances between  
568 mussel and fish farms) do not act as barriers to commercial IMTA (Alexander, 2015).

569

#### 570 4.4. Conclusions and future research considerations

571

572 This study demonstrates that; 1) extractive species cultivated in the vicinity of open-  
573 water fish farms experience a growth benefit due to integration and 2) close proximity of  
574 extractive species to the farm (0 m for macroalgae and 0-60 m for bivalves) can increase  
575 performance and therefore possibly profit but there appears to be some spatial flexibility  
576 around this if logistical constraints require it. Even though the extent at which nutrient  
577 extraction is carried out is still quantitatively unknown, spatially extensive locations of  
578 extractive species are known to significantly reduce organic loading around fish cages (Reid et

579 al, 2013a; Holdt & Edwards, 2014).

580 Future study recommendations include: 1) allocating control sites and locating these  
581 at suitable distances (>1 km to 8km) from fish farm effluent sources to avoid fish farm nutrient  
582 contamination; 2) including site details such as seawater parameters (e.g. temperature,  
583 salinity), hydrodynamics (current speeds and direction), food supply (chlorophyll-*a*, particulate  
584 organic matter and total particle matter concentrations), farm capacity (farm size and feeding  
585 protocols); and 3) determining the extent to which spatially extensive extractive species  
586 cultivation mitigates nutrient discharge from open-water fish farms (including consideration of  
587 the organic loading from the bivalve component of IMTA farms).

588 Open-water IMTA is still in development and further research can be expected to  
589 improve IMTA methodologies (e.g. optimum placings for extractive species and a wider range  
590 of commercial extractive species) leading towards more sustainable IMTA systems. Although  
591 complete (100%) nutrient sequestration is not practically feasible, future IMTA efforts should  
592 be encouraged given the environmental and economic merits of integration.

593

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597

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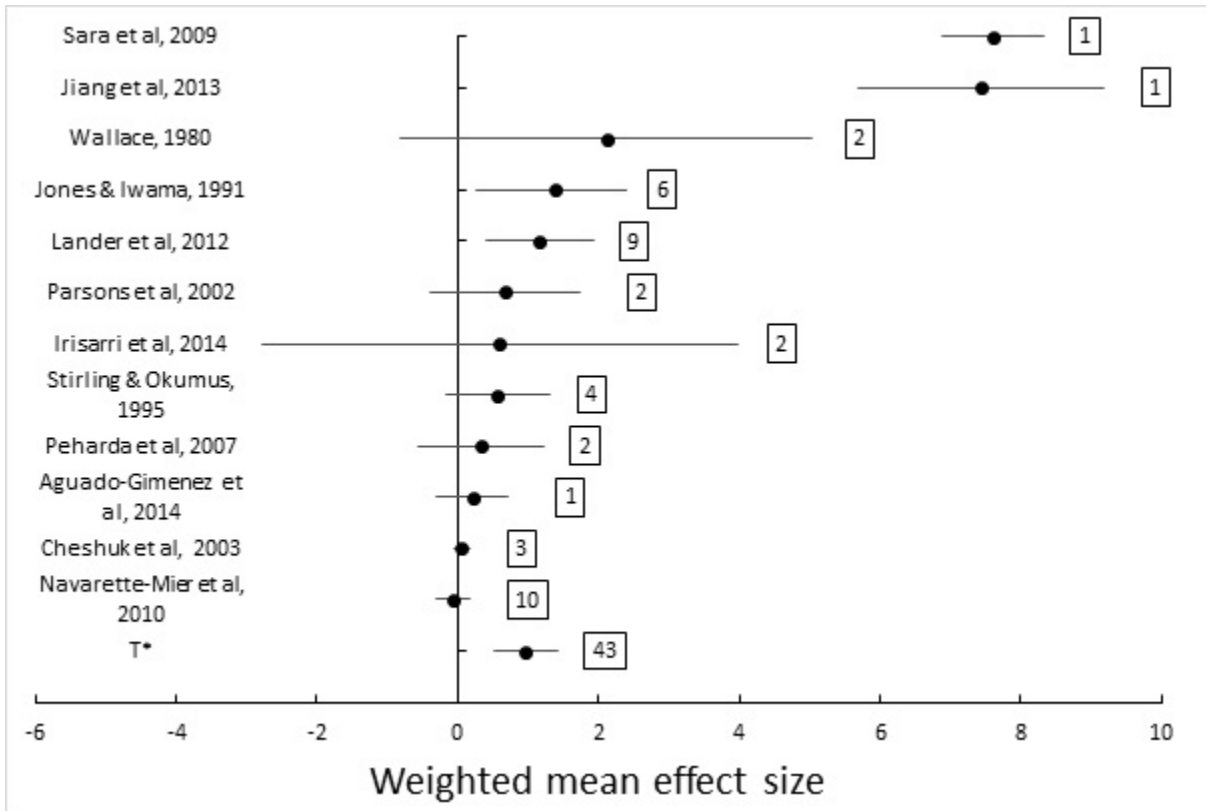
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986 **Figure legends**



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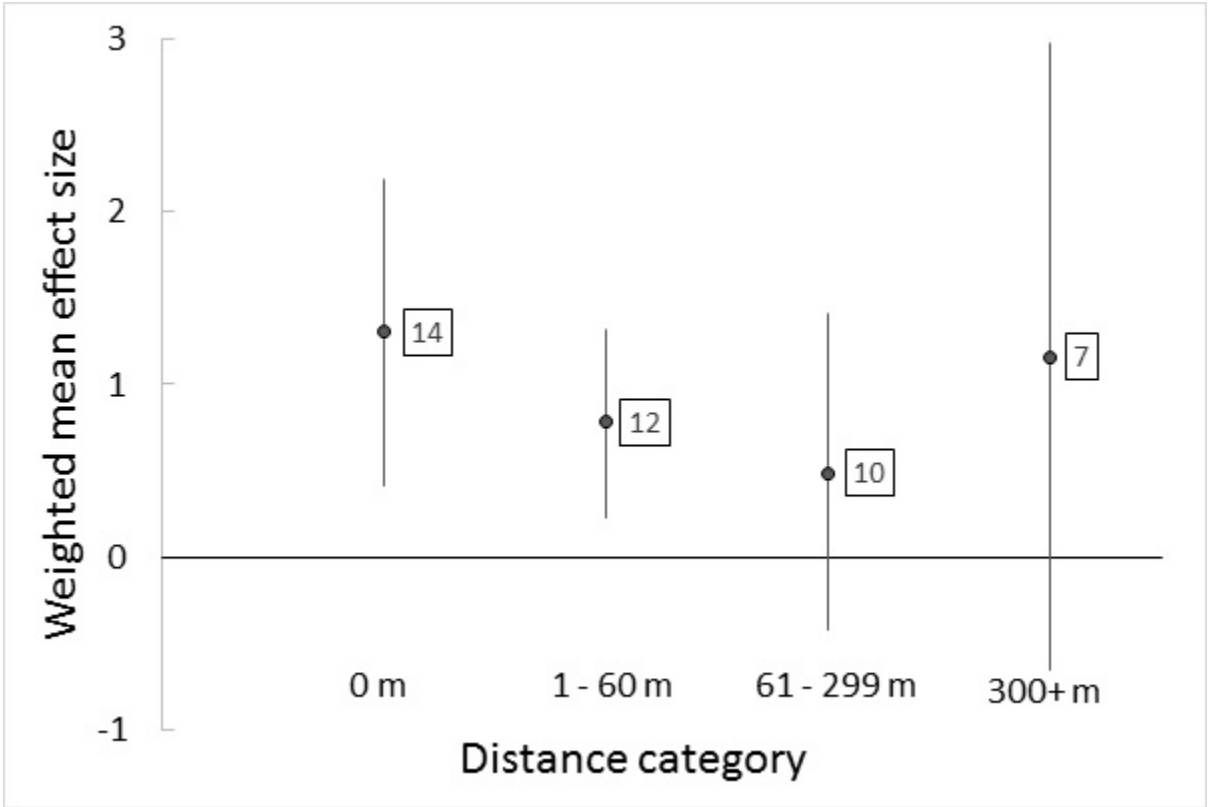
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**Figure 1.** Bivalve growth relative to controls (weighted mean effect size  $\pm$  95% confidence intervals) when integrated with open-water fish farms presented for individual studies and for all studies collated (total weighted mean effect size (T\*)). Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.



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**Figure 2.** Weighted mean effect size ( $\pm$  95% confidence intervals) for bivalves cultivated at varying distances from open-water fish farms. Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.

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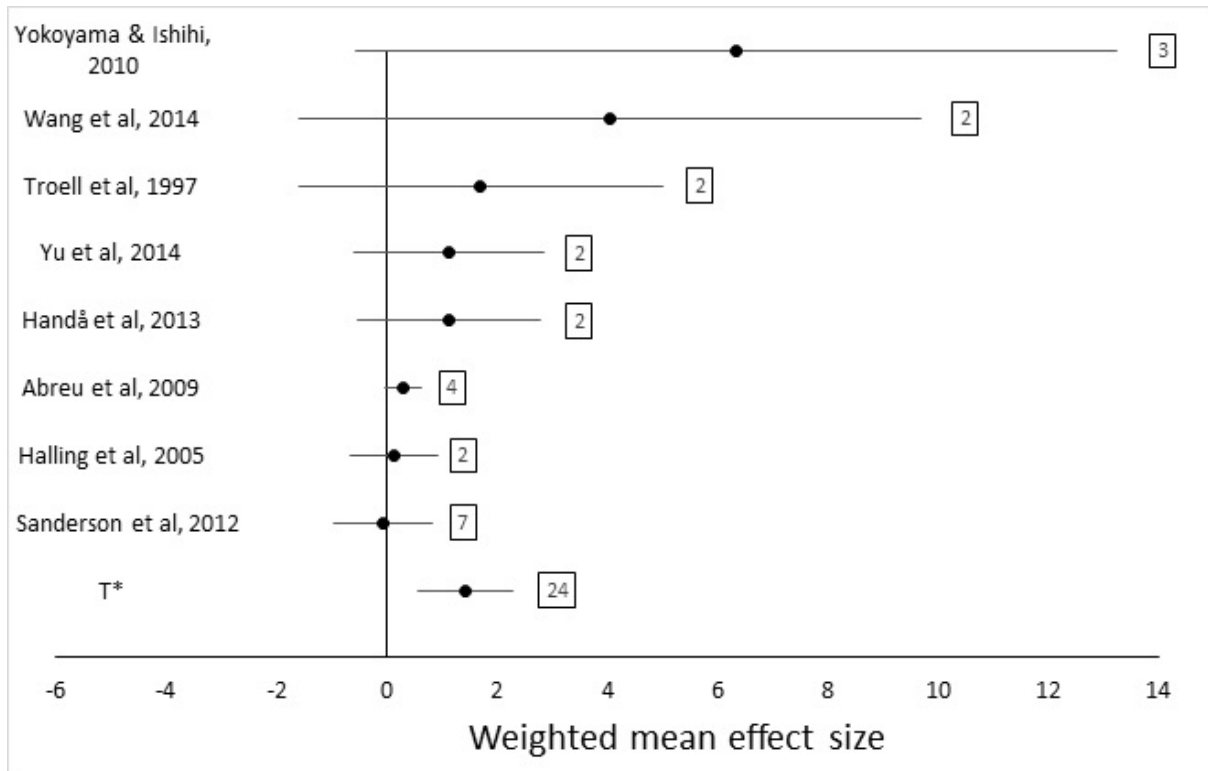
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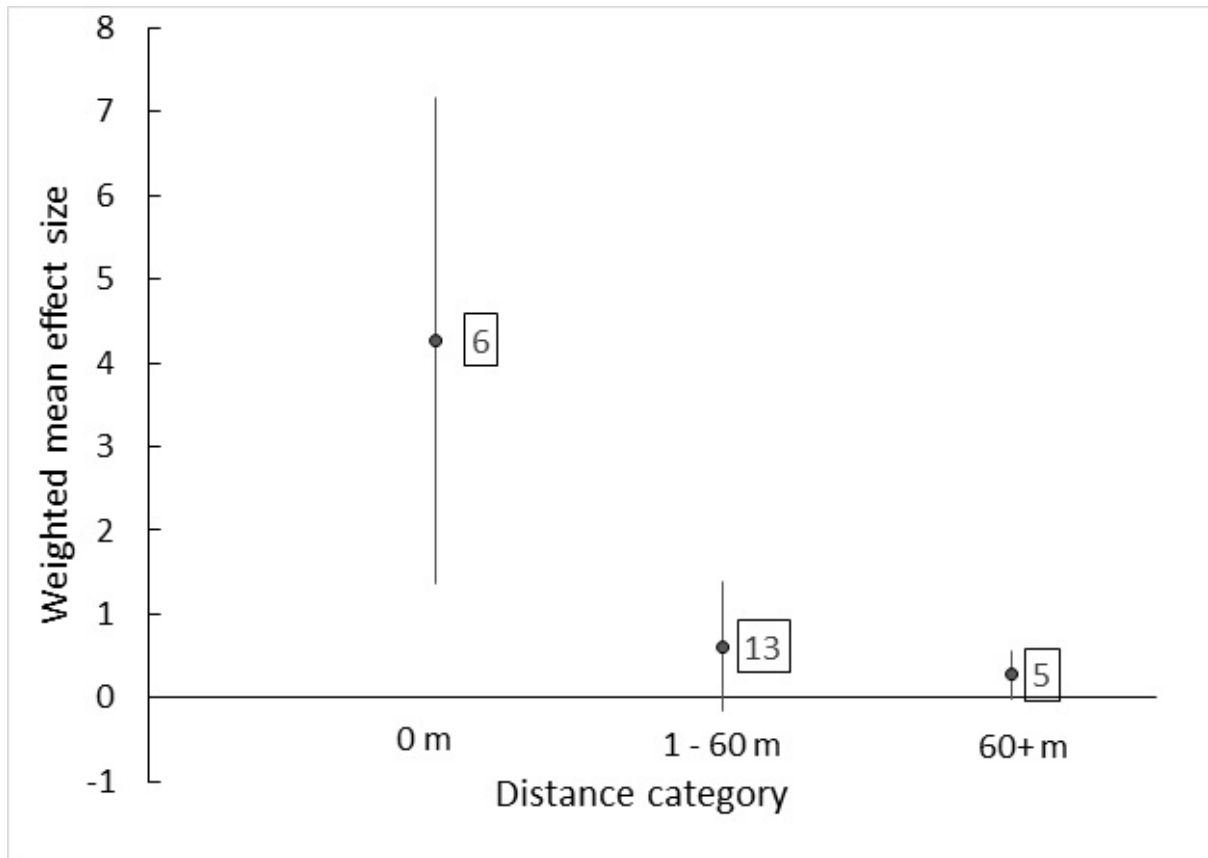
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**Figure 3.** Macroalgal growth relative to controls (weighted mean effect size  $\pm$  95% confidence intervals) when integrated with open-water fish farms presented for individual studies and for all studies collated (total weighted mean effect size (T\*)). Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.



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**Figure 4.** Weighted mean effect size ( $\pm$  95% confidence intervals) for macroalgae cultivated at varying distances from open-water fish farms. Boxed numbers represent the number of data points used to calculate weighted mean effect sizes.