**The impact of the sea anemone *Actinothoe sphyrodeta* on *Mytilus galloprovincialis*
mussel cultivation (Galicia, Spain)**

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22 **Abstract**

23 Marine mussel aggregations act as a substrate and refuge for many fouling species, which may attach
24 to mussel shells. Mussel cultivation in Galicia, Spain, is carried out on hanging ropes in subtidal
25 systems. The fauna associated with this cultivation includes a great number of invertebrates that
26 compete for space or food with the mussels, or use their clusters, as a refuge from predators or water
27 turbulence. Outbreaks of the epibiont anemone *Actinothoe sphyrodeta* have been reported in cultivated
28 Galician mussels since 2013, but their impact has not been scientifically investigated. Here, temporal
29 and spatial variability of *Actinothoe sphyrodeta* on mussel shells throughout one year is presented.
30 Sampling of mussel size, weight, and byssus attachment strength allowed calculation of mussel
31 tenacity (attachment strength relative to size). Higher *Actinothoe sphyrodeta* presence correlated with
32 lower mussel tenacity and greater biomass losses, suggesting that this species could be an
33 economically important biofouling component.

34 **Keywords:** *Mytilus galloprovincialis*, biofouling, tenacity, mariculture, *Actinothoe sphyrodeta*,
35 biomass losses

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51 **Introduction**

52 Shellfish aquaculture represents an important economic sector worldwide. There was a total of 17.1
53 million tons of mollusks produced in 2016, mainly clams, oysters and mussels, which are the most
54 commonly cultivated bivalve mollusks (FAO 2018). This important industry requires significant
55 infrastructure for its development, eg cultivation ropes, nets, or buoys, which together with the
56 cultivated organisms may offer appropriate surfaces for settlement or attachment by many different
57 species, a process known as biofouling, ie colonization of a living surface by sessile animals or plants
58 (see Wahl, 1989).

59 Fouling species are usually reported to have detrimental impacts on bivalve performance,
60 although some beneficial effects have been described. Higher epibiont presence is usually linked to
61 greater mortality and interference with mussel cluster formation (Garner and Litvaitis 2016). Lower
62 rates of growth and survival, and increased dislodgements due to excess weight have been reported
63 when epibionts are numerous (Witman and Suchanek 1984; Dittman and Robles 1991; Buschbaum
64 and Saier 2001; Thieltges and Buschbaum 2007; de Sá et al., 2007; Daigle and Herbingier, 2009).
65 Fouling can even change ecosystem function by modifying assemblages with multi-species
66 interactions that affect community diversity (Mazouni et al. 2001; Nizzoli et al. 2005; Giles et al.
67 2006; Wahl 2008). Among the mechanisms for negative impact on bivalves by fouling species,
68 highlighted is modification of the water flow, and so food and oxygen availability, which, together
69 with effects on valve opening capacity, can drastically change optimal bivalve performance (Wallace
70 and Reisnes, 1985; Arakawa, 1990; Lesser et al., 1992; Claereboudt et al., 1994; Taylor et al., 1997).
71 Beneficial impacts of biofouling can include protection of cultivated bivalves from predators or
72 harmful epibionts through secretion of bioactive compounds, or camouflage (Armstrong et al., 1999;
73 Ross et al., 2004; Farren and Donovan, 2007). Fouling may also increase primary production and food

74 availability to bivalves (Lodeiros et al. 2002; Ross et al. 2002; Le Blanc et al. 2003), or facilitate the
75 settlement of commercially farmed shellfish (Hickman and Sause 1984; Fitridge 2011).

76 Marine mussels are sessile organisms and secure themselves through an extracellular structure
77 called byssus, an array of collagenous threads secreted by the ventral groove of the foot (Waite 1992).
78 These byssal filaments allow mussels to form aggregations that may ameliorate the impact of highly
79 hydrodynamic environments or predators, and simultaneously increase fertilization success (Liu et al.
80 2011; Christensen et al. 2015). Byssus formation increases the heterogeneity and diversity of the
81 habitat, increasing substrate complexity and offering additional surface area for attachment by other
82 sessile organisms (Buschbaum et al. 2009), which is the basis for mussels being considered ecosystem
83 engineers (Borthagaray and Carranza 2007). Byssal threads decay over time (approximately 4 to 6
84 weeks) and must be continually replaced (Carrington 2002; Moeser and Carrington 2006). The latter
85 byssogenesis process can use 8–15 % of a mussel's total energy expenditure (Hawkins and Bayne
86 1985). Epibiont fouling has been reported to induce increases in both mussel byssal thread production
87 rate and byssal thread strength, as well as other factors like water flow and predation (Thieltges and
88 Buschbaum 2007; Babarro and Carrington 2011; Garner and Litvaitis 2013; Babarro et al. 2016; but
89 see O'Connor et al. 2006; O'Connor 2010).

90 In Galicia (North-West Spain), the marine mussel *Mytilus galloprovincialis* is cultivated in
91 coastal embayments, or rías, on floating rafts (20 m × 25 m) supporting 500 ropes, each 12 m long. A
92 combination of factors such as upwelling-favorable winds, phytoplankton availability, and coastal
93 morphology make the embayments of Rías Baixas exceptional sites for extensive culture of the mussel
94 *M. galloprovincialis*. Currently annual mussel production is approximately 236,022 tons, representing
95 around 12 % of worldwide production and 45 % of EU production (FAO 2016). The Ría de Arousa is
96 the largest ría, covering an area of 245 km² (Fig. 1 near here) and supporting 2404 mussel rafts.
97 Biofouling is generally dominated by sponges, barnacles, serpulid worms, ascidians and bryozoans
98 (reviewed in Dürr and Watson 2010). In Galician waters, the fouling fauna also include some hydroids,
99 algae, other bivalves, and anemones belonging to the class Anthozoa (Tenore and González 1975;

100 Román and Pérez 1979; 1982). Outbreaks of the sea anemone *Actinothoe sphyrodeta* (Gosse, 1858) in
101 cultivated Galician mussels, and their potentially causing mussel dislodgement were noted in early
102 summer 2013 but no directed research has been carried out. In this study, spatial and temporal
103 monitoring of *A. sphyrodeta* presence on cultivation ropes was carried out, and simultaneously mussel
104 size, weight and byssus attachment strength was measured to estimate biomass losses and calculate a
105 proxy for fragility of mussel attachment to cultivation ropes.

106 **Material and Methods**

107 ***Experimental design and sampling***

108 According to previous field observations in Ría de Arousa, *A. sphyrodeta* was only present at the outer
109 zones of the ría. Three sites of this embayment were chosen for monitoring: northern (OuN) and
110 southern (OuS) sites at the mouth (outer zone) of the ría and one central site (MidW, see Fig. 1). One
111 annual cycle, September 2015-July 2016, was monitored; covering the thinning out to harvest phase of
112 mussel cultivation (Pérez Camacho et al. 1991). Raft cultivation usually includes two phases: from
113 seeding to thinning out, and from thinning out to harvest. Three main reasons can be described for
114 selecting the thinning out to harvest phase: i) mussels are larger in this second phase (>50 mm shell
115 length), as is their shell area, the potential surface for biofouling settlers, ii) this cultivation phase
116 begins with uniform mussel size after a selection based on size, and more importantly removal of
117 biofouling fauna, iii) important dislodgement events usually occur during this period because mussels
118 are larger and heavier and therefore more prone to be dislodged from the ropes. Standard handling and
119 cultivation techniques were used for mussel deployment. Mussels of approximately 50 mm shell length
120 were used to begin experimental cultivation. Initial mean density values were 900 ± 49 mussels per
121 meter of rope. Special caution was taken to ensure that similar mussel density was used on all ropes, as
122 any significant difference could have influenced mussel growth responses after initial deployment
123 (Fréchette et al. 1996; Cubillo et al. 2012). Six twelve-meter length ropes separated by a distance of

124 least one meter were hung from the aft of a raft in each cultivation site. Sampling frequency was
125 approximately monthly, when all cultivation ropes were weighed for biomass data and one rope per
126 site used for cluster collection. One rope per site was sampled in each monthly sampling visit, and
127 there were 6 experimental cultivation ropes per site, so all ropes were sampled twice in the whole
128 experimental period (12 months). The second sampling of each rope occurred only after all 6 ropes
129 were sampled for the first time. Planned activities for each field sampling were ordered as follows: i)
130 weigh cultured ropes, ii) digitally photograph the mussel ropes over their entire length, iii) measure
131 attachment strengths of individual mussels, iv) cluster collection.

132 Environmental parameters were gathered from a local government agency (Instituto
133 Tecnológico para o Control do Medio Mariño, <http://www.intecmar.gal>) that has maintained an
134 extensive network of coastal oceanographic stations since 1992. Vertical water column temperature,
135 salinity and chlorophyll a (chl_a) data were taken from a Sea-Bird 25 CTD from stations closest to the
136 studied mussel rafts in the Ría de Arousa. Numerical configuration of the Regional Ocean Modelling
137 System model (Schepetkin and MacWilliams 2005) was used to estimate current velocity (CV) along
138 the water column in order to compute a food availability index (chl_a × CV). Wave parameters were
139 calculated from the forecast power spectra of the SWAN model (Simulating Waves Nearshore),
140 developed at Delft University of Technology, that computes random, short-crested wind-generated
141 waves on a curvilinear grid around the Galician coast, and was forced by the Meteogalicia operational
142 WW3 model (<http://www.meteogalicia.gal/web/index.action>). Mean values (± SD) of environmental
143 parameters (temperature, salinity, food availability, chlorophyll a, current velocity and wave height)
144 for the whole experimental period are included in Table 1.

145 ***Biomass in cultivation ropes: weighed vs. maximum weight***

146 Submerged rope biomass as live weight (kg) was monitored throughout the cultivation period using
147 two digital dynamometers (Jaguar™, with 0.1 kg precision and upper weight limits of 100 and/or 1000
148 kg; Industrias JAGUAR, S.A.L. Villabona, Guipúzcoa, SPAIN) and video recording (Nikon D3100;

149 Nikon Inc. NY, USA) to determine when stable weight values were obtained (~ 1 min, which exceeds
150 by far the maximum wave periodicity). Weight fluctuations over time (30 measurements in one
151 minute) were corrected by taking the geometric mean, which normalizes the ranges being averaged.
152 Cultivation ropes were also weighed in air once lifted onto the boat for handling. After taking digital
153 photographs and mussel tenacity measurements (see below), several mussel clusters along the rope
154 were sampled, corresponding to 3-4 % of the live weight of the rope, spaced by cultivation depth (1, 3,
155 5, 7, 9, and 11 m), then transported to the laboratory for later analysis. The live weight and number of
156 individuals in these clusters was recorded for each sampling date and site. Shell sizes were measured
157 to group mussels in size classes with 1 mm increments. Consequently, individual mussel weight could
158 be calculated after biofouling removal. Finally, the number of individuals and weight per cluster were
159 used to estimate the theoretical-maximum weight (mw) of the whole rope assuming no mortality or
160 dislodgements; $mw = d_{t0} \times (W / n)$, where d_{t0} is the initial density value, W is the weight of the clusters
161 sampled along the rope in kg, and n is the total number of mussels sampled in these clusters.
162 Accordingly, assuming that initial density value (900 individuals/m) remains constant throughout the
163 cultivation period, ie *zero* mortality and dislodgement from the ropes, the gap between the measured
164 rope weights and the theoretical maximum weights can be taken to estimate biomass lost (%).
165 Considering average mussel size, line weight (weighed vs. maximum) and starting density, theoretical
166 number of the mussels lost at each cultivation site could be obtained. Therefore, relative retention (%)
167 values were extrapolated as the amount of individuals that remained on ropes at the end of the
168 cultivation.

169 ***Image analysis: qualitative monitoring of Actinothoe sphyrodeta presence***

170 Digital photographs (Nikon D3100 with Nikkor 18-55 mm lens) were taken on the floating rafts after
171 raising up the full length of the cultivation ropes. The rope was divided into two sections, from 0-6 and
172 6-12 m length. Two photographs of the mussel clusters present at 1, 3, and 5 m (0-6 m) and 7, 9, and
173 11 m (6-12 m) were taken (n = 12 photographs per rope). Qualitative scores (0-4) for each photograph

were recorded for *A. sphyrodeta* presence according to the scheme in Fig. 2. An estimate of the percentage of mussel shell covered by the sea anemone for each qualitative score was obtained in the laboratory and varied from 0 % (0) to over 60 % (4). This percentage was estimated by analyzing images taken with a video camera (Leica IC A; Leica Microsistemas S.L.U. L'Hospitalet de Llobregat, SPAIN) on a stereo microscope (Leica MZ6) using the software QWin (© Leica Imaging Systems) on a computer (PC AMD Athlon XP 3000+; Orlando, Florida). Camera and light settings were set at the beginning of the analysis and kept constant throughout.

Mussel tenacity

At each sampling date, 60 individual mussels were dislodged from the cultivation rope sampled for cluster collection at 6 different depths (1, 3, 5, 7, 9, and 11 m; n = 10 mussels per depth) to estimate byssal attachment force (F, in Newtons) at different depths. Byssal strength of attachment was measured by connecting individual mussels from the cluster to a spring scale (Digital Force Gauge DN431, 0.01 N resolution; PCE Iberica S.L. Alicante, SPAIN) with the aid of custom-made forceps (Babarro and Comeau 2014). Mussels used in the dislodgement tests occupied the outer positions of the cluster, since such positions allowed easier access to the entire individual. Care was taken to avoid disturbing neighboring mussels when dislodging one individual. Mussels immediately adjacent to dislodged specimens were not measured because they are connected via byssus threads. Dislodgement measurements were made on wet mussels to prevent modification of the mechanical properties of the byssus. After dislodgement, the anterior-posterior (shell length), dorso-ventral (shell height) and lateral axes (shell width) of all mussels were measured with the aid of Vernier calipers (± 0.1 mm). Mussel tenacity (TEN, in $\text{N}\cdot\text{m}^{-2}$) was calculated by normalizing attachment force by the projected shell area of a dislodged individual: $\text{TEN} = F / \text{PA}$, where PA is the projected area of the individuals dislodged, approximately an ellipse obtained by the product of width and height values of shells, used as a characteristic index of mussel size for tenacity calculations (Bell and Gosline 1997). Tenacity values obtained for growing mussels throughout the cultivation period were normalized to a common size (60

199 mm shell length) following the formula: $TEN_{norm} = TEN_{exp} \times (60 / SL_{exp})^b$ where TEN_{norm} is the
200 normalized value, TEN_{exp} corresponds to the experimental value obtained for each monitored mussel
201 size, SL_{exp} is the size of the experimental mussels and b is the experimental allometric exponent that
202 relates tenacity and mussel size (shell length) obtained in the field ($b = -1.40$ [OuN, outer northern
203 site], -2.59 [OuS, outer southern site] and -1.83 [MidW, middle site], see Results section).

204 ***Statistical analysis***

205 The differences between observed and maximum rope weights assuming *zero* natural mortality due to
206 predation, self-thinning (Filgueira et al. 2008), and dislodgement for each cultivation site were fitted to
207 linear functions to estimate biomass lost during cultivation. Slopes of the linear functions at distinct
208 cultivation sites were tested for significant differences following the method by Zar (1999).

209 Variability in *A. sphyrodetta* presence was analyzed by ANCOVA with cultivation time as a
210 continuous variable, cultivation site and depth as independent factors, and experimental unit (rope
211 sampled) as a random variable. An exponential function was used to fit the increase in biomass losses
212 relative to sea anemone coverage of mussel shells.

213 Mussel tenacity values obtained throughout the cultivation period were fitted to potential
214 functions ($Y = aX^b$) relative to individual size where Y is tenacity and X is mussel shell length; a and b
215 are the corresponding intercept and slope values of the equation, respectively. The exponent value
216 obtained for each cultivation site (b) was used to normalize mussel tenacity to a 60 mm shell length
217 individual to enable direct comparisons between cultivation sites and depths. Two-way ANOVA was
218 used to explore the effect of both cultivation site and depth as factors on normalized tenacity. To
219 clarify data presentation, mussel tenacity was combined across the whole length of the rope (0-12 m)
220 because of the negligible effect of cultivation depth as an independent factor. ANCOVA was used to
221 test the differences between slopes and intercepts of the linear equations for mussel tenacity vs.
222 anemone density. Normality and homogeneity of variances were examined by the Shapiro-Wilk W -test
223 and Levene's test, respectively. All data was log-transformed when necessary, and if heterogeneity

224 persisted, rank transformation was used (Conover 2012). Significant differences between experimental
225 groups were identified using *a posteriori* tests (Bonferroni). All analyses were performed using
226 STATISTICA 7.0 software (StatSoft; TIBCO Software Inc. Palo Alto, CA USA) and SPSS Statistics
227 23 (IBM; IBM España S.A. Madrid, España). All data are reported as means $\pm$ SD.

228 **Results**

229 ***Biomass: observed vs. maximum***

230 Plotting observed vs. maximum weights of cultivation ropes at the three experimental sites generated
231 linear relationships (Fig. 3A). The linear model equations in the figure show that a significantly
232 shallower slope was obtained for mussels in the OuS site (0.367 ± 0.06 ; $p < 0.001$). In contrast, similar
233 (and steeper) slopes described the other cultivation sites (0.738 ± 0.05 and 0.795 ± 0.06 for OuN and
234 MidW, respectively; Fig. 3A). Divergence between linear functions was more pronounced in the
235 second half of the study period, February-July, during which there were larger mussel individuals with
236 greater shell surface areas to be colonized by potential epibionts, and more significantly, a higher
237 abundance of *A. sphyrodeta*. The final values for differences between weighed and maximum
238 biomasses at the end of cultivation were 9, 14, and 40 % for mussels cultivated in OuN, MidW and
239 OuS sites, respectively. Therefore, similar relative retention of 87-90% were obtained for OuN and
240 MidW sites whereas such value decreased to 60% for mussels cultivated at OuS site (Fig. 3B).

241 ***Actinothoe sphyrodeta: temporal-spatial variability and integration with biomass losses***

242 The presence of *A. sphyrodeta* on mussel shells across cultivation sites and depths throughout
243 experimental time is illustrated in Fig. 4A and B. ANCOVA analysis of anemone abundance with
244 cultivation time as a continuous variable, and cultivation site and depth as independent factors is
245 presented in Table 2. Time had a significant effect on the sea anemone abundance on mussel shells,
246 mostly driven by the higher values in spring and early-summer across all sites. Moreover, cultivation
247 site and depth explained significant differences in *A. sphyrodeta* abundance while there were no

significant interactions between factors (Table 2). Mussels cultivated at the OuS site showed relatively high values of *A. sphyrodeta* attachment on their shells throughout the whole cultivation period, with the second highest values found on mussels cultivated at the central MidW site (Fig. 4). In contrast, anemone presence on mussel shells at the OuN site was much lower throughout the year, but increased significantly towards the early-summer period (Fig. 4). In all cases, anemone presence decreased significantly in deep water (6-12 m) in compared to the more superficial layer (0-6 m) (Fig. 4A-B; Table 2). Mean values for this qualitative indicator of sea anemone presence on cultivation ropes across all depths (Fig. 4) was highest in the southern OuS and middle MidW sites (2.46-3.15 and 1.71-2.29 respectively), and minor anemone presence was detected at the OuN (0.19-0.32).

Exploring the relationship between sea anemone presence and biomass lost, illustrated in Fig. 5, it can be noted that values <3 for anemone presence resulted in a range of 2-5 % biomass loss. However, *A. sphyrodeta* presence >3 , which was observed mostly during the spring-summer period, corresponded with the highest biomass losses, ranging from 11-14 % at the MidW site and from 15-40 % at the OuS site (Fig. 5). Negative values obtained for biomass losses in case of anemone values <2 were removed and an exponential regression could be obtained for biomass losses vs. anemone abundance (Fig. 5).

Mussel tenacity

Byssus tenacity ($\text{N}\cdot\text{m}^{-2}$), or mussel attachment strength relative to size of individuals, through experimental time is presented in Fig. 6A. Generally, smaller mussels attached stronger than large mussels, as the data were described by exponential functions between tenacity and individual size (Fig. 6A). After normalizing tenacity to a standard value of shell length, 60 mm (Fig. 6B), the results of an ANOVA test revealed that tenacity of mussels at the OuN site was significantly higher, by approximately 30 %, than the lower (and similar) byssus tenacity values of mussels cultivated in the OuS and MidW sites (Table 3). Cultivation depth and interaction of depth $\times$ raft did not have a significant effect on mussel tenacity (Table 3). Mussel tenacity significantly decreased as anemone

273 presence increased at all cultivation sites (Fig. 6C). Despite being statistically significant, OuN was
274 removed from further analyses due to the small range of anemone abundance (most of them <1) and
275 the low explained variance (35.9 %) which could interfere with the comparison between cultivation
276 sites with high anemone abundance. ANCOVA performed on the linear functions that characterized
277 OuS and MidW populations showed no significant differences between slopes ($t = 1.562$; $df 18$;
278 $p > 0.05$) which suggests similar impact of the fouling organism on tenacity. However, statistically
279 different intercepts show that mussels attach stronger at the OuS site of cultivation as compared to the
280 MidW site ($t = 3.939$; $df 19$; $p < 0.001$; Fig. 6C) which means that other factors may play a role in
281 mussel tenacity.

282 **Discussion**

283 The presence of biofouling on mussel cultivation ropes can cause detrimental effects on commercial
284 production and consequently it is highly relevant to mussel farmers. Previous surveys in the study area,
285 Ría de Arousa, during the 1970-80s regarding fauna associated with mussel cultivation did not
286 mention *A. sphyrodetta* anemone presence in a total number of 84-99 species of invertebrates, though it
287 may have been within the category ‘undetermined Anthozoa’ (Tenore and González 1975; Román and
288 Pérez 1979; 1982). According to these early studies, colonization of cultivation ropes may have
289 occurred because of propagules from adjacent ropes and the settlement of pelagic larvae during the
290 year. Although these studies represent a significant analysis of associated fauna from a taxonomic
291 point of view, monitoring of epibionts on cultivated species became of greater interest when they were
292 found to negatively impact operational practices or cause biomass loss (Antoniadou et al. 2013).
293 Summarizing some of these early studies, Pérez Camacho et al. (1991) reported that natural mortality
294 for thinned-out mussels in the same area of the present research, would be 14-20 %, although potential
295 detachment events of mussels from ropes due to storms were not considered, which would have
296 increased biomass losses, as noted by the authors.

297 Relatively similar environments in terms of hydrodynamics, eg wave height values, between
298 the northern (OuN) and southern (OuS) outer mouths of the ría (Table 1), supported very distinct
299 mussel responses with regards to byssus tenacity and biomass loss (Figs. 5-6) that could have been
300 driven by *A. sphyrodetta* presence. In this model, the low relative retention of mussels on cultivation
301 ropes (60%) eg larger biomass losses for OuS mussels could be explained by the low tenacity of
302 individuals at this site compared to the hydrodynamically similar OuN site, which would be a
303 consequence of the higher *A. sphyrodetta* presence and its negative impact on mussel attachment
304 strength as reported in this study.

305 When comparing mussel response between the outer OuS site with the central MidW site, both
306 with high anemone presence, hydrodynamics may be the major driver of differences in tenacity and
307 biomass loss. ANCOVA performed on mussel tenacity vs. anemone variability highlighted similar
308 slopes which mean that the impact of this fouling organism on mussel attachment strength in these two
309 populations was similar. Accordingly, slight differences in mean mussel tenacity between these two
310 stations (OuS and MidW) did not match major differences in hydrodynamic energy. Therefore,
311 although *A. sphyrodetta* presence is high at the MidW site, especially at the end of the experimental
312 cultivation, the much calmer MidW site would have prevented high biomass losses. Accordingly, the
313 significantly different intercepts between these populations would suggest that anemone abundance is
314 not the only driver for tenacity, but other factors such as mussel size and/or hydrodynamic
315 environment could produce tenacity differences and therefore different detachment rates.

316 Comparing the maximum biomass losses observed at OuS in the present survey (40 %) and that
317 reported for natural mortality within the same geographical area (20 %, Pérez Camacho et al. 1991), a
318 similar impact can be inferred for fouling interference and natural mortality on biomass. One
319 interesting aspect concerning mussel cultivation in Galicia is that mussel density normally used by
320 farmers is relatively high (900/m in this survey) which may decrease the probability of heavy
321 colonization by fouling species of cultivation ropes, which are already fully occupied by mussels

(competition by space). However, such mussel density may create an ideal alternative surface, in the mussel shells themselves, for fouling organisms to attach and take advantage of the local environment.

This study is the first empirical evidence of negative impact on commercial mussel cultivation by the anemone *A. sphyrodeta*. *A. sphyrodeta* is a predatory species that feeds mostly small fishes and zooplankton with the actions of its tentacles (Sebens et al. 2016). Therefore, competition for food resources can be negligible for this anemone-mussel interaction since mussels are filter-feeders with phytoplankton as main food type resource. However, like other fouling organisms (eg barnacles, kelp) anemones may establish a three-dimensional structure on mussel shells that can produce an excess of weight (Fritidge et al. 2012). In our case, excess of weight would not be significant as compared to other fouling organisms like barnacles or kelp that may affect the probability of dislodgement by increasing hydrodynamic loading (Witman and Suchanek 1984; Dittman and Robles 1991; O'Connor et al. 2006; Thieltges and Buschbaum 2007). Punctual measurements of such weight ratio between anemone abundance and mussel cluster in the spring-early summer period (high presence of the fouling organism) gave a maximum value of 2.5% (or less). *In situ* observations have revealed that sea anemone under study secrete a mucus-type exudate through their epidermis with adhesive properties that allows them to occasionally make small movements on shells (Wotton 2004). High levels of secretion in the case of high anemone abundance on cultivation ropes, eg >3 according to the scheme in Fig. 2A, could make free-living mussels more prone to being dislodged, not by excess weight but by inability to secrete optimal byssus filaments to attach within clusters. This statement is based on a preliminary microcosm experiment in which free living mussels forced to attach to slates covered by the anemone, *A. sphyrodeta*, were unable to secrete byssus filaments at similar rates to those on uncovered slates (see Appendix A). This response would be indicative of a repelling surface for new byssal thread secretion and attachment. Therefore, integration of mussel tenacity with byssus secretion, biomass losses and *A. sphyrodeta* presence would allow understanding of the cascade of events associated with this epibiont.

347 Studies of the fauna associated with mussel cultivation ropes ensure better understanding of the
348 relationships established between organisms, and the mechanisms behind particular events of special
349 interest to the industry. Here proposed is a model of how a sessile species of anemone (*A. sphyrodeta*)
350 may interfere with the mussel strategy of attachment on ropes and eventually cause potential biomass
351 losses that exceed natural mortality reported for the area by a factor of two (Pérez Camacho et al.
352 1991). According to Fitridge et al. (2012), fouling organisms encountered on aquaculture shell surfaces
353 can cause the following negative consequences for cultured species: physical damage, mechanical
354 interference with feeding actions, competition for food and space, changes in the environment and
355 culture infrastructure, eg reduced water flow, oxygen or food availability, and/or increased weight on
356 cultured stock and equipment (see Introduction for other references). With the probable exceptions of
357 the first (physical damage) and last (significant weight increase) potential impacts, the anemone *A.*
358 *sphyrodeta* could exert the remaining potential negative effects, resulting in greater production costs
359 and losses of cultivated biomass. Further research is needed to test these hypotheses. As an example,
360 any potential effect of high *A. sphyrodeta* presence on optimal mussel valve opening must be tested in
361 future work. The mussel *M. galloprovincialis* in Galicia interacts almost permanently with its
362 environment since its valves are open 97.5 % of the time (Comeau et al. 2018). Any restriction on this
363 opening behavior by the interference of *A. sphyrodeta* or other macrofouling species could also affect
364 mussel behaviors like byssus secretion by foot extension, filtration activity, or respiration, which could
365 have consequences on growth, fitness, and the ability of mussels to remain attached to cultivation
366 ropes.

367 Relationships between cultivated resources and associated fauna and their eventual impacts on
368 yield and sustainability are of increasing interest (Lacoste and Gaertner-Mazouni 2015) and new or
369 updated monitoring programs should lead to a better understanding of the causes and consequences of
370 macrofouling. There is a requirement to evaluate the economic costs of biofouling due to biomass loss
371 and its spatial and temporal variability in cultivation areas, and therefore predict where and when
372 cultured individuals are more prone to be lost. These types of surveys would need to be implemented

373 with continuous monitoring of mussel responses, characterization of associated fauna, and
374 measurement of inter-annual variability. The negative impact of biofouling on cultivated mussel
375 biomass reported in this survey may become even more significant in the near future under global
376 climate change.

377 The presence of the sea anemone *A. sphyrodetta* may be a significant driver of biomass loss in
378 mussel culture; however, further studies are necessary to investigate the underlying environmental
379 factors behind spatial and temporal variation in fouling presence and which biotic and/or abiotic
380 factors play important roles in such variability as well as in the performance of cultivated mussels.
381 Potential shifts in fouling distribution between outer and inner areas of rías or estuaries where
382 aquaculture activities are widely developed, will be of great importance for the near future. Fouling
383 impacts are difficult to avoid (Fitridge et al. 2012), therefore understanding temporal and spatial
384 variability of this and other fouling species will be crucial for increasing knowledge of their impacts
385 and may inform effective avoidance strategies.

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390 **Declaration of interest statement**

391 The authors declare no conflict of interest.

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[Figure captions]

Figure 1. Map of the study area showing the locations where mussels were cultivated at the outer section of Ría de Arousa (OuN and OuS in blue and red, at the northern and southern mouths of the ría, respectively; MidW in purple at the middle section of the ría).

Figure 2. (A) Scheme used for the qualitative analysis of the sea marine anemone *Actinotheroe sphyrodeta* on mussel clusters under raft cultivation. *A priori* established values ranged between 4 (highest coverage of mussel shells, >60%) and 0 (complete absence). In parenthesis are shown the corresponding values for such coverage of the anemone occupying mussel shell area as settlement surface. (B) the sea anemone *Actinotheroe sphyrodeta* covering commercial mussel ropes in Ría de Arousa.

Figure 3. (A) Linear functions for the relationship between observed and maximum weights of mussel ropes (in Kg) at different cultivation sites. Squares, crosses and triangles represent the northern OuN in blue, the southern OuS in red and middle MidW in purple sites, respectively. (B) Relative retention (%) as indicative of the amount of mussels that remained on cultivation ropes at the end of the experimental period for each cultivation site.

Figure 4. Presence of the sea anemone *Actinotheroe sphyrodeta* as a qualitative indicator (means $\pm$ sd) at different cultivation sites for a year cycle and the two distinct water depths (0-6 m, A and 6-12 m, B). Values for each experimental site varied between 0 and 4 for complete absence and highest abundance (see Material and Methods), respectively. Mean values for the whole experimental period are included in the Figure for each cultivation site.

Figure 5. Relationship between the presence of the sea anemone *A. sphyrodeta* and biomass gap (%) between observed and maximum weights (in kg) for the two cultivation sites where the anemone represent significant values (at least > 1 for most of the cultivation period).

Figure 6. (A) Mussel tenacity variability in relation to shell length increase of individuals with cultivation time. (B) Mean mussel tenacity values ($\pm$ sd) normalized for a standard 60-mm shell length individual during the whole cultivation period (September 2015-July 2016). See Material and Methods for normalization equation. (C) Mussel tenacity variability with regard to anemone presence on mussel clusters.

Figure 1

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Figure 4

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673 Figure 6

Table 1. Mean ($\pm$ sd) values for each location of the Ría de Arousa during the studied period for temperature (T), salinity (S), food availability (FA), chlorophyll a (Chla), current velocity (CV) and wave height (WH).

Raft	T	S	FA	chla	CV	WH
	°C		$\text{mg} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	$\text{mg} \cdot \text{m}^{-3}$	$\text{m} \cdot \text{s}^{-1}$	m
OuN	14.43 (1.58)	34.41 (1.06)	0.45 (0.33)	2.94 (3.54)	0.15 (0.09)	1.23 (0.56)
OuS	14.51 (1.51)	34.54 (1.01)	0.28 (0.21)	1.90 (2.28)	0.15 (0.09)	1.50 (0.63)
MidW	14.54 (1.49)	34.53 (0.73)	0.22 (0.17)	1.59 (1.83)	0.14 (0.09)	0.68 (0.39)

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Table 2. General linear model analysis of covariance to determine the effects of time (T) as a continuous variable and cultivation site (raft, R) and depth (D, m) as factors on anemone presence on mussel shells. Individual ropes, the experimental unit (ExpUnit, U), were included as a random factor. Dependent variable was rank-transformed prior to the analysis. ns, not significant ($p>0.05$).

anemone presence			
Factor	df	F	P
time (T)	1	73.026	<0.001
raft (R)	2	448.079	<0.001
depth (D)	1	24.089	<0.01
ExpUnit (U)	5	0.767	ns
R x D	2	1.972	ns
R x U	10	0.853	ns
D x U	5	2.036	ns
R x D x U	10	0.343	ns
Error	27		

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Table 3. Two-way ANOVA for the byssus tenacity variability of a 60-mm shell length standard mussel as a function of cultivation site (raft, R) and depth (D, m) with responses of individuals during the whole experimental period. See main text (Material and Methods) for specific details of each factor. Dependent variable was rank-transformed prior to the analysis. ns, not significant ($p>0.05$).

Byssus tenacity			
Factor	df	F	P
raft (R)	2	30.158	<0.001
depth (D)	1	2.585	ns
R x D	2	0.032	ns
Error	59		

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