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3 **Quantifying imperfect detection in an invasive pest fish and the implications for**
4 **conservation management**

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26

27 ABSTRACT

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29 In managing non-native species, surveillance programmes aim to minimise the
30 opportunity for invasions to develop from initial introductions through early detection.
31 However, this is dependent on surveillance methods being able to detect species at
32 low levels of abundance to avoid false-negative recordings through imperfect
33 detection. We investigated through field experimentation the ability to detect
34 *Pseudorasbora parva*, a highly invasive pest fish in Europe, in relation to their known
35 density and sampling method. Secure pond mesocosms of area 100 m² contained *P.*
36 *parva* densities from 0.02 to 5.0 m⁻²; each density was in triplicate. These were
37 searched using point sampling electric fishing and deployment of fish traps (non-
38 baited and baited). No fish were captured at densities < 0.5 m⁻² using any method and
39 this was considered their detection threshold. Point sample electric fishing was the
40 least effective detection method, producing high proportions of false-negative data
41 even at high fish densities. Baited traps were the most effective detection method.
42 Probability of detection of *P. parva* was 1.0 for baited traps at all densities > 0.5 m⁻²,
43 whereas for electric fishing it only exceeded 0.95 at 5.0 m⁻² using high searching
44 effort. These data reveal that small pest fishes such as *Pseudorasbora parva* may be
45 prone to imperfect detection when at low densities and this is consistent with a
46 number of other invasive species. This indicates the importance of designing
47 surveillance programmes using methods of known statistical power to optimise
48 conservation resource expenditure and enhance management outcomes.

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50 **Keywords:** Non-native species; detection threshold; electric fishing; false-negative;
51 probability of detection.

52 **1. Introduction**

53

54 Biological invasions are a conservation issue that have the potential to negatively
55 impact biodiversity and raise global concern over biotic homogenisation (McKinney
56 and Lockwood, 1999). Consequently, a common goal of the conservation
57 management of biological invasions is removing invasive ‘pest’ species and then
58 keeping areas pest-free (Moore et al., 2010). To be effective, adequate resources
59 require to be apportioned to surveillance in order to increase the opportunity for new
60 introductions to be detected (Moore et al., 2010; Britton et al., 2010a). Early detection
61 then enables management actions to be taken in the incipient phases of invasion that
62 inhibit establishment and minimise dispersal rates that should impede or even prevent
63 invasion (Hulme, 2006; Christy et al., 2010; Willson et al., 2010). However, this is
64 reliant on the surveillance methods being capable of capturing the species when they
65 are in low abundance, with ‘imperfect detection’ referring to situations when
66 introduced individuals have not been able to be detected (Rout et al., 2009a,b).
67 Imperfect detection also inhibits the evaluation of eradication operations; in these
68 situations, ‘false-negative’ data must be minimised in order to reduce the chance of
69 errors occurring in its evaluation (Simberloff, 2003; Rout 2009; Rout et al., 2009a,b;
70 Delaney and Leung, 2010).

71

72 Knowledge on the chance of imperfect detection occurring during either
73 surveillance or eradication evaluation is enhanced when the detection probability of
74 the species and sampling methods are quantified. Low probability of detection of
75 invasive species has been recognised across a number of taxonomic groups, including
76 mustelids (King et al., 2009), snakes (Willson et al., 2010) and plants (Rout, 2009),

77 and has enabled probability models to be developed (Harvey et al., 2009; Rout et al.,
78 2009a,b; Christy et al., 2010). The utility of these models is they enable the
79 development and optimisation of search strategies (Cacho et al., 2006), reduce the
80 chance of false-negative data being collected (Rout et al., 2009a), and enable better
81 prioritisation of conservation resources within management programmes (Delaney
82 and Leung, 2010).

83

84 Imperfect detection can be a general issue when surveying fish populations due to,
85 for example, inefficiencies that arise from issues of fish size and water depth that
86 inhibit capture (Janac and Jurajda, 2005; Cowx et al., 2001; Copp et al., 2010).
87 Quantification of imperfect detection has not, however, been applied to the
88 conservation management of non-native fishes, despite the potential of their invasions
89 to cause substantial negative impacts in the environment (Gozlan et al., 2010a). These
90 invasion issues are well demonstrated by the Asian cyprinid fish topmouth gudgeon
91 *Pseudorasbora parva* (Temminck and Schlegel). Highly invasive in Europe since the
92 1960s, they are now present in at least 32 countries (Gozlan et al., 2010b).
93 Introductions generally occur through inter-country movements of fish in the
94 aquaculture trade, with their release into the wild via small, outdoor aquaculture
95 ponds that are connected to open waters (Britton et al., 2007, 2008, 2010b). In
96 colonised waters, they are generally considered pests due to their numerical
97 dominance and small body sizes (25 to 90 mm fork lengths; Pinder et al., 2005;
98 Britton et al., 2007; Gozlan et al., 2010b). Ecological impacts include their sharing of
99 trophic space with native fishes resulting in decreased growth (Britton et al., 2010c)
100 and the transmission of a novel pathogen (Gozlan et al., 2009). Whilst considerable
101 effort and resource has been exerted in some European countries to control their

102 invasion (Britton et al., 2008; Britton et al., 2010b), the evaluation of eradication
103 operations is inhibited by a paucity of knowledge on their probability of detection (i.e.
104 the opportunity for collecting false-negative data). Moreover, there are no known
105 active surveillance programmes for this fish at the present time, despite extensive
106 work on their dispersal and associated impacts (Gozlan et al., 2010b).

107

108 Consequently, the aim of this work was to quantify the imperfect detection of
109 introduced *P. parva* in experimental pond systems in relation to known population
110 density, sampling method and search effort. This was achieved through comparing the
111 efficacy of different sampling approaches in detecting their presence in order to
112 produce an unbiased estimate of their probability of detection. This was completed in
113 replicated mesocosm systems through experimentally manipulating the sampling
114 method, search effort and fish densities. A model was then produced to estimate the
115 probability of detecting an individual *P. parva* at different densities and levels of
116 sampling effort. Given the paucity of data on the probability of detection of invasive
117 pest fish generally then these outputs will have management applications to other pest
118 fishes. They should also provide an important conservation case study in the utility of
119 applying imperfect detection to improve management programmes of invasive
120 species.

121

122 **2. Materials and Methods**

123

124 *2.1 Experimental design and search methodology*

125 A series of secure, replicated semi-natural mesocosm ponds of 100 m² and maximum
126 depth 1.5 m were set up in June 2010. These were designed to broadly represent the

127 outdoor aquaculture ponds that *P. parva* are typically introduced to when transferred
128 in the aquaculture industry (*cf.* Section 1). These mesocosms were located on a bio-
129 secure aquaculture site where *P. parva* were already present and established. In each
130 mesocosm, the initial step was to place a 4 m² keep cage containing 5 live *P. parva*
131 for 24 hours. As all fish survived, these cages were removed and *P. parva* of fork
132 lengths 60 to 80 mm introduced into the mesocosms at densities of 0.02, 0.1, 0.5, 1.0,
133 1.7, 2.7 and 5.0 m⁻². Each density was replicated 3 times. The mesocosms were then
134 left for 14 days to allow the *P. parva* to acclimatise to the conditions before searches
135 commenced.

136

137 Surveillance of the mesocosms for completing the searches of *P. parva* was
138 completed in July and August 2010. The searches commenced using non-quantitative
139 point sampling electric fishing (Copp, 2010) using a Smith Root LR24 backpack
140 fisher operating at approximately 0.5-A pulsed DC, where the conductivity of each
141 pond was in the region of 350 to 400 μ s. Electric fishing was preferred to the use of a
142 micromesh seine net (Cowx et al. 2001) as submerged vegetation would have
143 prevented the net's effective use. Point sampling was preferred to continuous electric
144 fishing as this enabled less-disruptive sampling within the different habitats of the
145 mesocosms (e.g., in- and outside of macrophyte cover) and enabled strong
146 quantification of fishing effort. Moreover, electric fishing in this manner has been
147 deployed to detect *P. parva* in the evaluation of their eradication operations (Britton et
148 al., 2010b). Each mesocosm was sampled once per week over a 3 week period. On
149 each occasion, the mesocosms were all sampled for the detection/ non-detection of *P.*
150 *parva* through the electric fishing of 30 randomly selected point samples where each
151 point was fished for a standard period of 10 s (Copp, 2010). Detection/ non-detection

152 of *P. parva* was recorded according to whether any individuals were observed within
153 the electric field. At the conclusion of sampling each mesocosm on each occasion,
154 turbidity was assessed using a Secchi disk (nearest cm), surface weed cover estimated
155 (to the nearest 5 %) and the time of day recorded. Throughout the sampling period,
156 the water temperature in each mesocosm was recorded every 30 minutes to the nearest
157 0.1 °C using a temperature logger.

158

159 Following completion of the electric fishing, each mesocosm was sampled using a
160 rectangular fish trap with a circle alloy frame of length 107 cm, width and height 27.5
161 cm, mesh diameter 2 mm and with funnel shaped holes of 6.5 cm diameter at either
162 end to allow fish entry and hence their capture. The traps were set either as non-
163 baited, i.e. no attractant was added to the trap, or baited, where 5 fishmeal pellets of
164 21 mm diameter were placed in the trap as an attractant (Dynamite Baits 2010). The
165 traps were fished on 6 occasions, with 3 days between each fishing occasion, with 1
166 trap set in each mesocosm for 1 hour. The sampling schedule of the mesocosms was
167 designed randomly to minimise bias, but was set up to ensure each mesocosm was
168 sampled on 6 occasions covering sampling using 3 non-baited and 3 baited traps.
169 Recording of the detection/ non-detection of *P. parva* in each mesocosm was
170 determined by their presence/ absence in the trap at the conclusion of the hour. To
171 minimise handling of the fish, they were able to be released without the traps being
172 removed fully from the water. The exception was on the final sampling occasion
173 when the traps were removed from the water and the captured fish counted to enable
174 trap catch per unit effort to be expressed as the number of captured fish per trap hour
175 ($n \text{ trap}^{-1} \text{ h}^{-1}$).

176

177 2.2 Statistical testing and probability of detection

178 The initial step in testing the search data was to determine the detection threshold for
179 each sampling method, expressed as the minimum density at which at least 1 *P. parva*
180 was detected by the method on at least 1 occasion. Comparisons were then made to
181 identify the effectiveness of each method to provide a presence/ absence search
182 methodology for *P. parva* and then their ability to provide measures of relative
183 abundance. This latter comparison was completed by comparing the number of
184 sampled fish with their known density and testing using regression methods. Multiple
185 regression was then used to identify the relative effects of fish density and the other
186 measured variables (weed cover (%), turbidity, time of day and water temperature) on
187 catch per unit effort. Their effects were compared using their standardized beta
188 coefficients (β) and their significance; those variables with the largest β values made
189 the strongest singular contribution to explaining the relative abundance (the dependent
190 variable) when all the other model variables were controlled.

191

192 As per Delaney and Leung (2010), logistic regression was used to test for a
193 relationship between the probability of detection of at least one individual in a
194 mesocosm its POD measured as the binary yes (detection) or no (non-detection),
195 against the number of point samples (up to the maximum of 30) or trap hours required
196 to detect that fish (S) and the density of *P. parva* (n), where a , b and c were the
197 regression coefficients, and where the compliment of POD is the probability of a
198 false-negative: $POD = e^{(a+bn+cS)} / 1 + e^{(a+bn+cS)}$ (Equation 1; Delaney and Leung, 2010).
199 From this model, the sampling intensity required to detect defined *P. parva* densities
200 at given PODs was determined and displayed using a contour plot.

201

202 3. Results

203

204 The detection threshold for *P. parva* for all sampling methods was 0.5 m^{-2} , i.e. no fish
205 were detected at densities of 0.02 and 0.1 m^{-2} and so were considered as false-
206 negative data (Fig. 1). Electric fishing and non-baited traps continued to produce a
207 proportion of samples that were false-negative even at densities of 5 m^{-2} whereas
208 baited traps did not produce any false-negative data at densities $> 0.5 \text{ m}^{-2}$ (Fig. 1).

209

210 At densities above 0.5 m^{-2} , electric fishing was only able to estimate *P. parva*
211 apparent presence/ absence; even in mesocosms of higher *P. parva* density, detection
212 generally involved observing a single individual in the electric field. Overall, catches
213 of *P. parva* were significantly lower in non-baited traps than baited traps at all
214 densities where fish were captured (ANOVA $F_{1,5} = 298.1$; $P < 0.01$; Fig. 2). Whilst
215 the non-baited fish traps did provide a significant relationship between *P. parva*
216 density and relative abundance (Fig. 2), this relationship for the baited fish traps was
217 highly significant (Fig. 2). The calibration equation for determining *Pseudorasbora*
218 *parva* density (n) from catch per unit effort values (c) in the baited traps was $n = (c \times$
219 $0.027) + 0.0106$. In the non-baited and baited traps, multiple regression analysis
220 revealed that *P. parva* density was the only significant variable in explaining the
221 variation in the catch per unit effort data (Table 1). Turbidity (Secchi disk depth range
222 0.3 to 0.6 m), weed cover (range 25 to 55 %), water temperature (range 17.1 to 17.6
223 $^{\circ}\text{C}$) and the time of day the samples were taken (range 08.30 to 16.30) had no
224 significant effects (Table 1). For baited traps, the variables in the model explained
225 most of the variation in the catch per unit effort data ($R^2 = 0.89$; Table 1); whilst this

226 was reduced in the non-baited traps ($R^2 = 0.64$), this may be related to their weaker
227 relationship between fish density and catch per unit effort (Fig. 2).

228

229 The multiple logistic regressions revealed that both *P. parva* density (n) and the
230 number of electric fishing point samples (S) had a significant effect on POD; this was
231 also similar for the non-baited fish traps (Table 2). For the baited fish traps, the trap
232 hours required for detection (S) was not significant ($P > 0.05$; Table 2) as at densities
233 $> 0.5 \text{ m}^{-2}$, the setting of 1 baited trap for 1 hour resulted in positive detection. Use of
234 the regression coefficients in Equation 1 enabled the probability of detection to be
235 determined as a function of sampling effort (number of point samples/ trap hours
236 required for detection) and *P. parva* density for each sampling method. For baited
237 traps, the probability of detection using a single trap for 1 hour at all densities > 0.5
238 m^{-2} (their detection threshold) was 1.0. For non-baited traps, the probability of
239 detecting *P. parva* at 0.5 m^{-2} was 0.78 in 1 trap hour, 0.94 in 2 trap hours and 0.99 in 3
240 trap hours. At densities $> 0.5 \text{ m}^{-2}$, POD was > 0.95 in 1 non-baited trap hour. The
241 POD of point sampling electric fishing was more complex, as revealed by the contour
242 plot produced from the model coefficients (Table 2) that plotted POD as a function of
243 point sample number and *P. parva* density (Fig. 3). Probability of detection only
244 exceeded 0.95 at 5.0 m^{-2} when at least 30 point samples were taken. At a density of
245 0.5 m^{-2} , POD only exceeded 0.95 when 54 point samples were taken.

246

247 **4. Discussion**

248

249 It has been argued that there is a local and global requirement to address biological
250 invasions and this will be assisted by enhanced surveillance methods that specifically

251 target the detection of newly introduced species (McKinney and Lockwood, 1999;
252 Hulme, 2006). Indeed, efforts to limit incursions by newly introduced species tend to
253 be preferable to conducting eradication operations over large spatial areas through
254 reduced expense and increased opportunity for success (Jarrad et al., 2010). Thus,
255 quantifying the probability of detection according to methodology, search effort and
256 the abundance of the target species is a fundamental step in identifying the optimal
257 design of a surveillance programme. Moreover, the increasing importance of
258 designing surveillance methodologies that incorporate known statistical power is
259 demonstrated in Australia where the Western Australian Government has imposed a
260 condition that surveillance programmes must include a specified statistical power in
261 order to detect newly introduced species (80 %; Jarrad et al., 2010). Consequently, the
262 conservation relevance of the outputs of this study are three-fold: (i) it should enable
263 the optimal design of surveillance and eradication evaluation surveys for invasive *P.*
264 *parva* and similar small bodied, invasive fishes using methodologies of quantified
265 statistical power; (ii) it has experimentally quantified the statistical power of detection
266 for a highly invasive species when other studies have had to rely on either simulations
267 (e.g. Tyre et al., 2003) or field observations (e.g. King et al., 2009); and (iii) it reveals
268 in a different taxonomic group to similar studies (e.g. Harvey et al., 2009; King et al.,
269 2009; Rout, 2009; Willson et al., 2010) that the issue of imperfect detection remains
270 inherent within invasive species management and so has to be firmly embedded
271 within surveillance and eradication programmes.

272

273 In designing surveillance strategies for new and extant populations of invasive
274 species, the collection of presence/ absence data only (rather than abundance) is
275 increasing (Delaney and Leung, 2010). This is because it potentially provides greater

276 spatial coverage for the amount of resource applied and statistical approaches for
277 testing these data are becoming increasingly powerful (Tyre et al., 2003; Wintle et al.,
278 2005; Delaney and Leung, 2010). It is, however, only relatively recently that
279 searchers have started account for the possibility of imperfect detection in surveillance
280 (Harvey et al., 2009), despite their serious implications in the (mis)interpretation of
281 search data (Rout et al., 2009a,b; Delaney and Leung, 2010). In the mesocosms used
282 here, false-negative data were always collected when fish density was below 0.5 m^{-2} .
283 This may have been a symptom of low random encounter probabilities due to the
284 limited number of replicated mesocosms and the low number of fish therein.
285 However, subsequent work in other mesocosms of $< 0.5 \text{ m}^{-2}$ using baited traps
286 deployed for 24 hours also produced false-negative data, despite studies in other
287 animals showing the duration of trap deployment increases the opportunity of capture
288 (e.g. Gust and Inglis, 2006). Consequently, these data do suggest that newly
289 introduced *P. parva* are unlikely to be detected in their incipient phases and may only
290 be detected at higher densities when natural dispersal may have already occurred and
291 the opportunity for taking effective management is constrained (Simberloff, 2003;
292 Hulme, 2006; Gozlan et al., 2010b). This does represent a serious management issue
293 and highlights the requirement for increased quarantine procedures that aim to prevent
294 introductions rather than for a new introduction to be detected. Indeed, such data
295 should be used to assist the determination of the optimal allocation of resources
296 between quarantine and surveillance through providing data on search efficiency and
297 its subsequent cost (Moore et al., 2010).

298

299 The choice of surveillance method for detecting new introductions remains a key
300 component of determining whether the species will be captured or if a false-negative

301 result is achieved. Whilst we demonstrated that baited traps were the most effective
302 method to detect *P. parva* in ponds, their utility in rivers of moderate flow or above
303 may be lower and electric fishing may be more appropriate, particularly across large
304 spatial areas. Given the issues of reduced probability of detection of *P. parva* when
305 using point abundance electric fishing, then alternative methods may require
306 exploring that were not covered by this study, such as use of more continuous electric
307 fishing over more extended periods, or use of micro-mesh seine nets in areas of
308 preferred *P. parva* habitat (cf. Beyer *et al.*, 2007). Indeed, whilst the search strategy
309 deployed in this study was geared around identifying the most appropriate rapid
310 detection tool for lentic *P. parva* populations, search effort remains a key component
311 within surveillance and increased effort by electric fishing (point-sampling or
312 continuous) may have provided increased probability of detection. Notwithstanding,
313 inefficiency of capture remains commonplace for many invasive animals through
314 issues such as trap avoidance, low detection opportunity due to low numbers of
315 individuals across large spatial areas and use of cryptic habitats inhibiting capture
316 (Dorcas and Willson, 2009; King *et al.*, 2009; Willson *et al.*, 2010). Consequently,
317 this has to be recognised in their management and accounted for whenever possible.

318

319 In summary, this study quantified the imperfect detection of *P. parva* in small pond
320 systems, similar to those ponds used in aquaculture where initial introductions of the
321 species into new spatial areas often occur. It revealed that management programmes
322 that rely heavily on detecting invasive species at low densities may be inhibited by
323 imperfect detection. It suggests that the issue of collecting false-negative data in
324 surveillance and eradication evaluation programmes is as apparent in a small invasive
325 pest fish as with many other invasive taxa, demonstrating that quantifying the

326 statistical power of detection methods should substantially improve the design of
327 search strategies and so enhance long-term conservation outcomes.

328

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330

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333

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Table 1. Multiple regression output of catch per unit effort of (a) non-baited and (b) baited traps versus the variables of *Pseudorasbora parva* density, weed cover (expressed as % of surface area), turbidity (measured as Secchi disk depth), time of day and water temperature that the sample was taken. See Section 3 for the data range of each variable between the mesocosms.

(a) Non-baited traps

Overall model: $R^2 = 0.64$; $F_{5,30} = 10.47$; $p < 0.01$

Variable	β (standardised)	P
Fish density	0.83	< 0.01
Weed cover	0.56	0.21
Turbidity (as Secchi disk depth, cm)	0.35	0.43
Time of day	0.34	0.54
Water temperature	0.01	0.74

(b) Baited traps

Overall model: $R^2 = 0.89$; $F_{5,30} = 24.35$; $p < 0.01$

Variable	β (standardised)	P
Fish density	0.69	< 0.01
Weed cover	-0.41	0.24
Turbidity (as Secchi disk depth, cm)	0.15	0.64
Time of day	0.13	0.14
Water temperature	0.11	0.18

Table 2. Multiple logistic regression coefficients used in equation 1, and their statistical significance, for the sampling approaches of point abundance electric fishing, non-baited fish traps and baited fish traps.

Sampling method	Parameter	Symbol in equation 1	Coefficient	Standard error	<i>P</i>
Point sampling electric fishing	Constant	a	3.19	0.85	< 0.03
	Fish density	b	0.56	0.15	< 0.02
	Point samples	c	0.11	0.03	< 0.01
Non-baited fish traps	Constant	a	21.25	7.54	< 0.05
	Fish density	b	42.12	19.59	< 0.01
	Trap number	c	1.54	0.54	< 0.04
Baited fish traps	Constant	a	15.49	4.21	< 0.03
	Fish density	b	55.12	22.14	< 0.01
	Trap number	c	2.10	1.92	> 0.05

Figure captions

Figure 1. Relationship of detection success (proportion of sampling occasions that resulted in the detection of *Pseudorasbora parva*) and *Pseudorasbora parva* density by sampling methods, where: × point sample electric fishing, ● non-baited fish traps, and ○ baited fish traps. Error bars represent 95 % confidence limits.

Figure 2. Relationship of mean catch per unit effort of non-baited (●) and baited (○) fish traps for *Pseudorasbora parva* at known densities in the 100 m² mesocosms. Error bars represent 95 % confidence limits. The solid line represents the significant linear relationship between fish density and catch per unit effort for the baited traps ($R^2 = 0.99$; $F_{1,60} = 274.4$, $P < 0.001$) and the dashed line for the significant relationship between fish density and catch per unit effort for the non-baited traps ($R^2 = 0.74$; $F_{1,60} = 174.4$, $P < 0.01$)

Figure 3. Contour plot of predicted probability of detection (POD) versus known densities of *Pseudorasbora parva* in 100 m² mesocosms and the number of electric fishing points required for detection. Key: i: < 0.19; ii: 0.20 - 0.39; iii: 0.40 - 0.59; iv: 0.60 - 0.79; and v) 0.80 - 0.95.

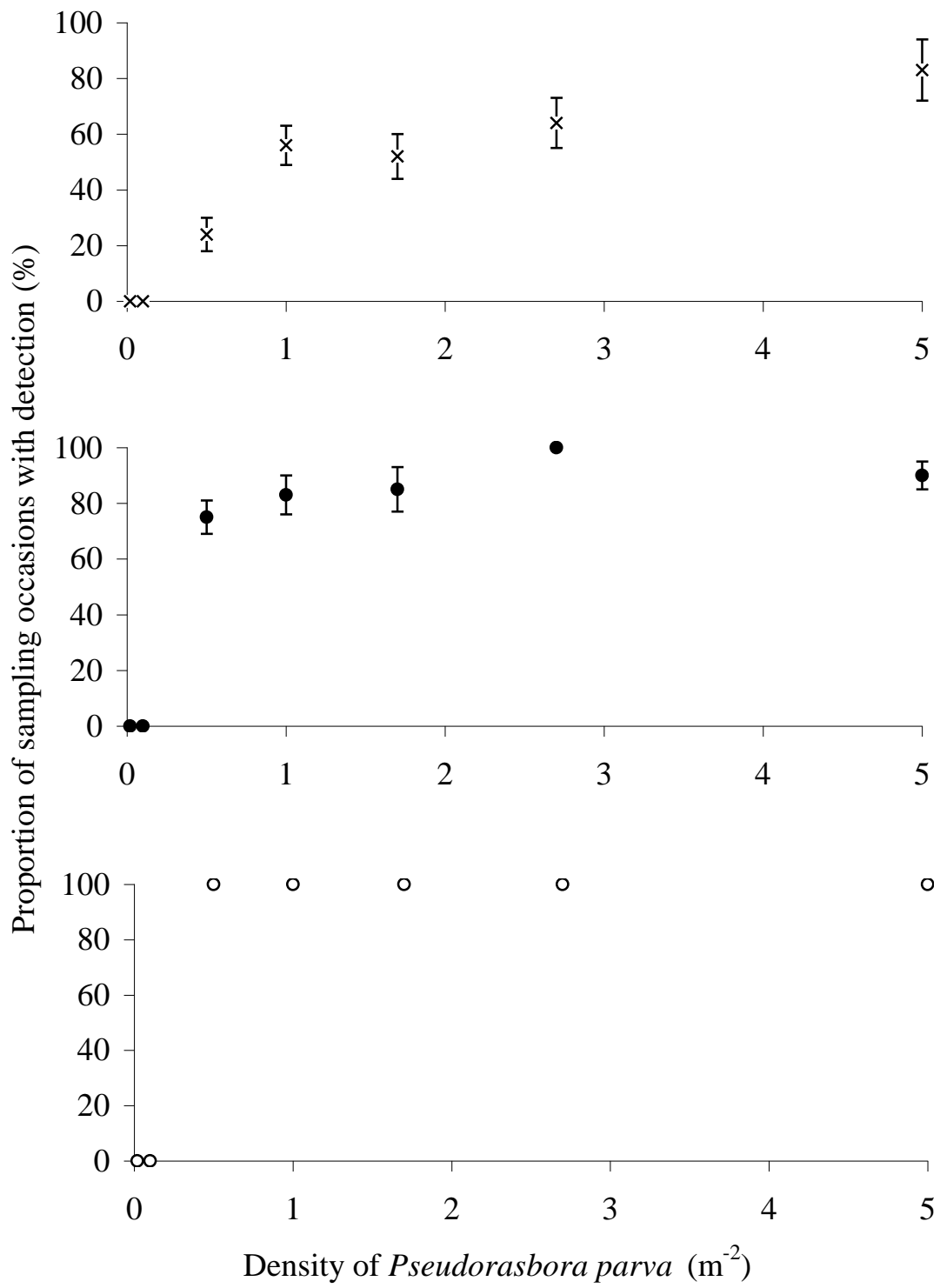


Figure 1.

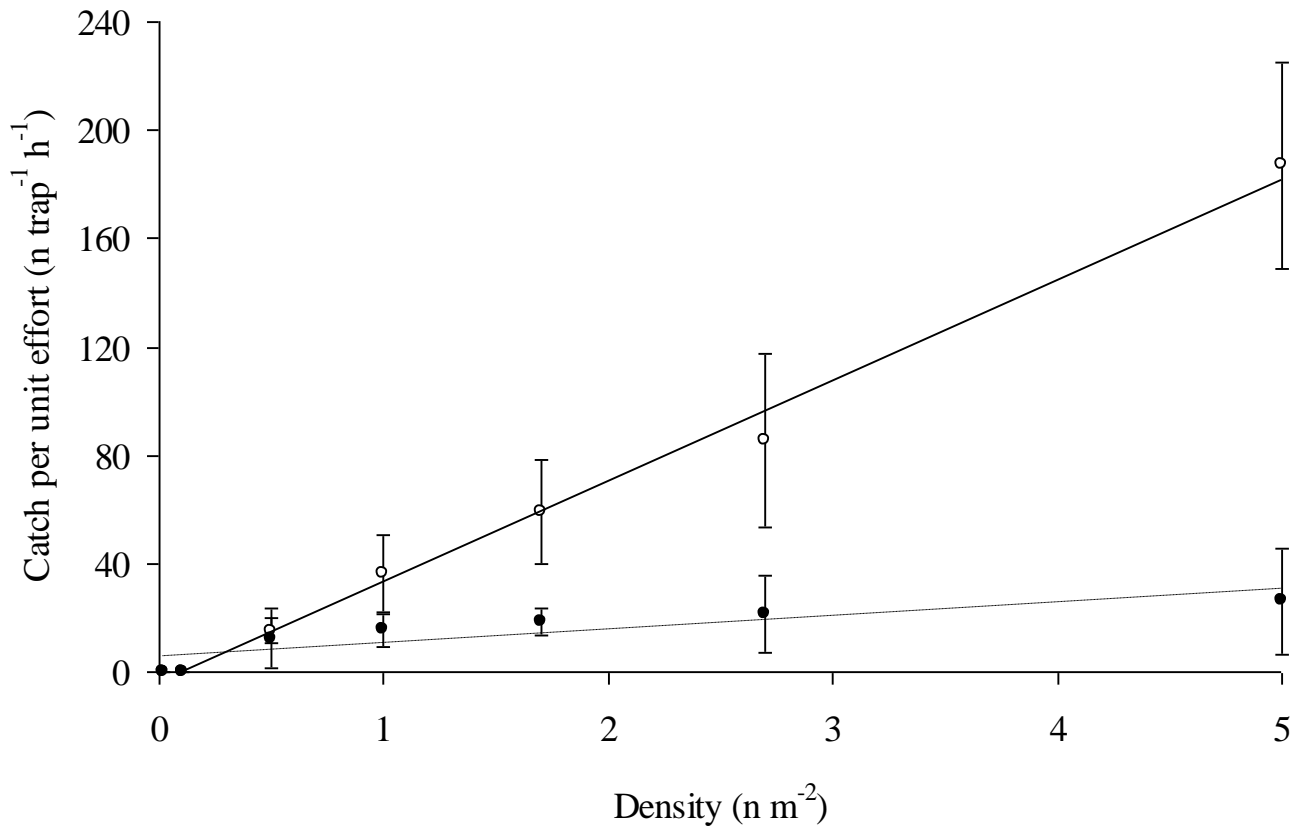


Figure 2.

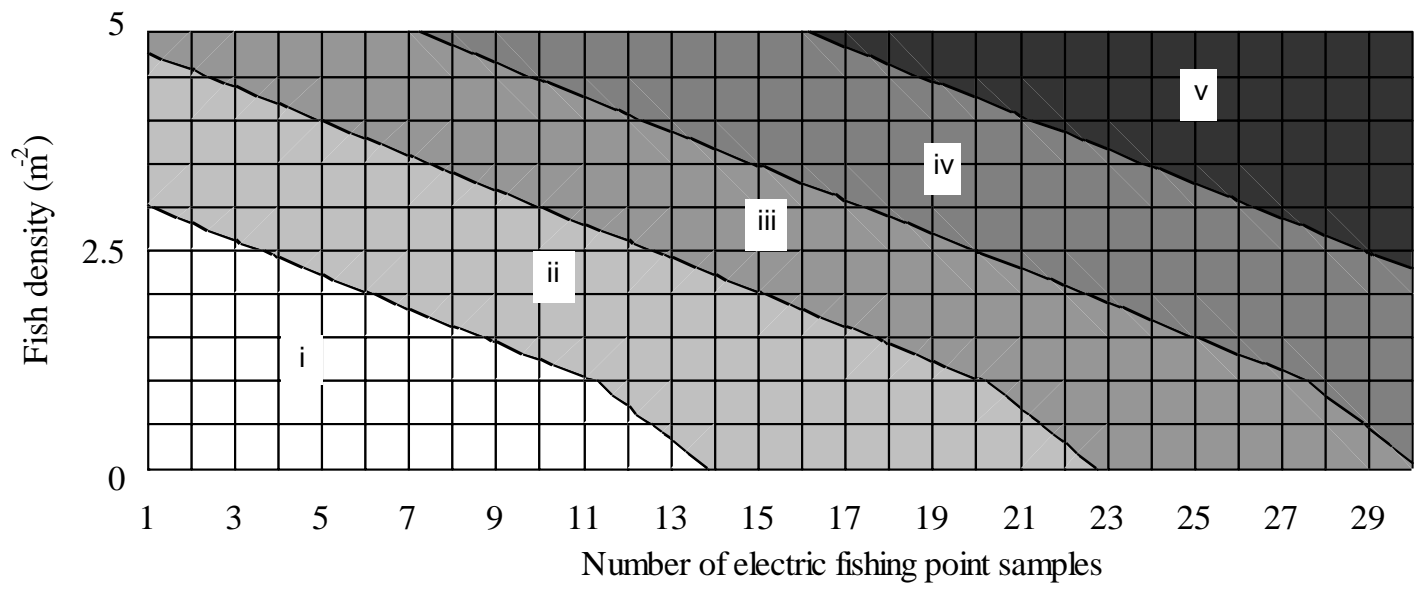


Figure 3.