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

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In managing non-native species, surveillance programmes aim to minimise the opportunity for invasions to develop from initial introductions through early detection. However, this is dependent on surveillance methods being able to detect species at low levels of abundance to avoid false-negative recordings through imperfect detection. We investigated through field experimentation the ability to detect Pseudorasbora parva, a highly invasive pest fish in Europe, in relation to their known density and sampling method. Secure pond mesocosms of area 100 m<sup>2</sup> contained P. parva densities from 0.02 to 5.0 m<sup>-2</sup>; each density was in triplicate. These were searched using point sampling electric fishing and deployment of fish traps (nonbaited and baited). No fish were captured at densities < 0.5 m<sup>-2</sup> using any method and this was considered their detection threshold. Point sample electric fishing was the least effective detection method, producing high proportions of false-negative data even at high fish densities. Baited traps were the most effective detection method. Probability of detection of *P. parva* was 1.0 for baited traps at all densities  $> 0.5 \text{ m}^{-2}$ , whereas for electric fishing it only exceeded 0.95 at 5.0 m<sup>-2</sup> using high searching effort. These data reveal that small pest fishes such as *Pseudorasbora parva* may be prone to imperfect detection when at low densities and this is consistent with a number of other invasive species. This indicates the importance of designing surveillance programmes using methods of known statistical power to optimise 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.

#### 1. Introduction

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

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

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

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Imperfect detection can be a general issue when surveying fish populations due to, for example, inefficiencies that arise from issues of fish size and water depth that inhibit capture (Janac and Jurajda, 2005; Cowx et al., 2001; Copp et al., 2010). Quantification of imperfect detection has not, however, been applied to the conservation management of non-native fishes, despite the potential of their invasions to cause substantial negative impacts in the environment (Gozlan et al., 2010a). These invasion issues are well demonstrated by the Asian cyprinid fish topmouth gudgeon Pseudorasbora parva (Temminck and Schlegel). Highly invasive in Europe since the 1960s, they are now present in at least 32 countries (Gozlan et al., 2010b). Introductions generally occur through inter-country movements of fish in the aquaculture trade, with their release into the wild via small, outdoor aquaculture ponds that are connected to open waters (Britton et al., 2007, 2008, 2010b). In colonised waters, they are generally considered pests due to their numerical dominance and small body sizes (25 to 90 mm fork lengths; Pinder et al., 2005; Britton et al., 2007; Gozlan et al., 2010b). Ecological impacts include their sharing of trophic space with native fishes resulting in decreased growth (Britton et al., 2010c) and the transmission of a novel pathogen (Gozlan et al., 2009). Whilst considerable effort and resource has been exerted in some European countries to control their invasion (Britton et al., 2008; Britton et al., 2010b), the evaluation of eradication operations is inhibited by a paucity of knowledge on their probability of detection (i.e. the opportunity for collecting false-negative data). Moreover, there are no known active surveillance programmes for this fish at the present time, despite extensive work on their dispersal and associated impacts (Gozlan et al., 2010b).

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

## 2. Materials and Methods

- 124 2.1 Experimental design and search methodology
- 125 A series of secure, replicated semi-natural mesocosm ponds of 100 m<sup>2</sup> and maximum
- depth 1.5 m were set up in June 2010. These were designed to broadly represent the

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

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

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

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

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#### 2.2 Statistical testing and probability of detection

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

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

#### 3. Results

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

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

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

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

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## 4. Discussion

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

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

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

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

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

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In summary, this study quantified the imperfect detection of *P. parva* in small pond systems, similar to those ponds used in aquaculture where initial introductions of the species into new spatial areas often occur. It revealed that management programmes that rely heavily on detecting invasive species at low densities may be inhibited by imperfect detection. It suggests that the issue of collecting false-negative data in surveillance and eradication evaluation programmes is as apparent in a small invasive 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. 328 329 Acknowledgements 330 331 The work was supported by the Natural Environment Research Council (NERC 332 research grant reference number NE/H000429/1). 333 334 **REFERENCES** 335 336 Beyer, K., Copp, G.H., Gozlan, R.E., 2007. Microhabitat use and interspecific 337 associations of introduced topmouth gudgeon Pseudorasbora parva and native 338 fishes in a small stream. Journal of Fish Biology 71 (SD), 224-238. 339 Britton, J.R., Davies, G.D., Brazier, M., Pinder, A.C., 2007. A case study on the 340 population ecology of a topmouth gudgeon *Pseudorasbora parva* population in the 341 UK and the implications for native fish communities. Aquatic Conservation: 342 Marine & Freshwater Ecosystems 17, 749-759. 343 Britton, J.R., Davies, G.D., Brazier, M., Chare, S., 2008. Case studies on eradicating 344 the Asiatic cyprinid *Pseudorasbora parva* from fishing lakes in England to prevent 345 their riverine dispersal. Aquatic Conservation: Marine & Freshwater Ecosystems 346 18, 867-876. 347 Britton, J.R., Gozlan, R.E., Copp, G.H., 2010a. Managing non-native fish in the environment. Fish and Fisheries DOI: 10.1111/j.1467-2979.2010.00390.x. 348 349 Britton, J.R., Davies, G.D., Brazier, M., 2010b. Towards the successful control of 350 Pseudorasbora parva in the UK. Biological Invasions 12, 25-31.

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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 mesoscosms.

# (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	Coefficient	Standard	P
		equation 1		error	
Point sampling	Constant	a	3.19	0.85	< 0.03
electric fishing	Fish density	b	0.56	0.15	< 0.02
	Point samples	c	0.11	0.03	< 0.01
Non-baited fish	Constant	a	21.25	7.54	< 0.05
traps	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 o baited fish traps. Error bars represent 95 % confidence limits.

Figure 2. Relationship of mean catch per unit effort of non-baited (•) and baited (o) fish traps for *Pseudorasbora parva* at known densities in the 100 m<sup>2</sup> 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  $100 \text{ m}^2$  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.

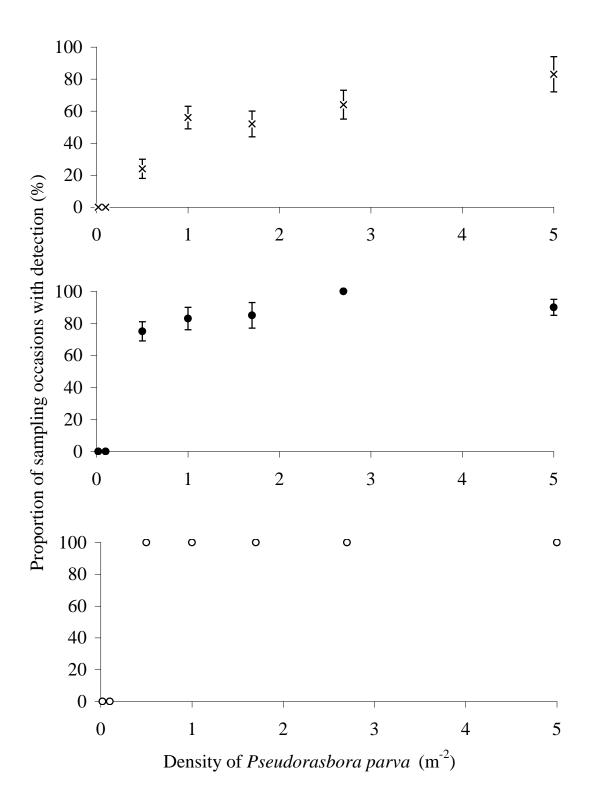


Figure 1.

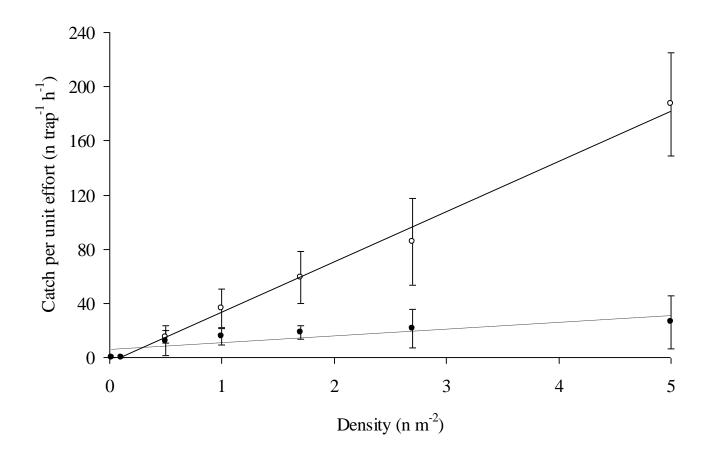


Figure 2.

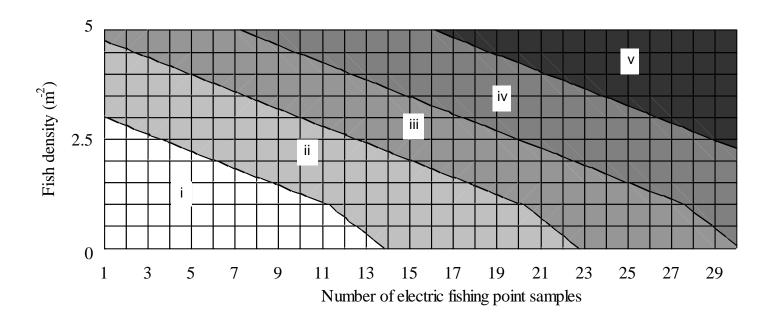


Figure 3.