Research Article

Assessing the Population Status of a Tree Species Using Distance Sampling: *Aquilaria crassna* (Thymelaeaceae) in Northern Laos

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Lack of reliable and accurate field data affect assessments of population status of tree species, especially tropical taxa with broad distributions. Use of distance sampling techniques may help to overcome the problem. This paper describes a method for estimation of absolute density of a rare tree species with scattered and clumped distribution, using line transect distance sampling. The method was applied to previously harvested populations of *Aquilaria crassna* Pierre ex H. Lec. (Thymelaeaceae) at four sites in Northern Laos. This species is destructively harvested to yield agarwood, probably the world's most valuable nontimber forest product and categorised as "critically endangered". The average density of felled *A. crassna* trees at the four sites was 2.2 ha^{-1}, indicating that harvesting has been extensive. However, the estimated densities of living saplings, 10.9 ha^{-1}, small trees (DBH < 10 cm), 10.6 ha^{-1}, and larger trees (DBH ≥ 10 cm), 1.7 ha^{-1}, suggest that populations are not wiped flat. The survey method should stand a good chance of wider use as a tool in management and conservation of a wide range of tree species. Results of the present case study could suggest that the conservation status of *A. crassna* should be re-categorised.

1. Introduction

According to a global assessment, roughly 9,000 tree species are threatened with extinction [1] and many species have therefore become the focus of increasing conservation concern [2]. However, the sources of information used for such assessments are generally limited to flora and taxonomic revisions, herbaria collections, and inventory and mapping exercises [1]. The quality and detail of data from these sources may be insufficient to accurately assess the status of a particular species, especially tropical taxa with broad distributions [3], and may cause erroneous assessments and misguided conservation efforts.

Tree species of the genus *Aquilaria* (Thymelaeaceae) are instructive cases of the difficulties and challenges in assessing population status and harvesting impacts [3]. *Aquilaria* populations are widespread but clumped and occur at low densities [3–5]. Furthermore, reduction of habitat area and excessive, indiscriminate harvesting for agarwood [6], probably the world's most valuable nontimber forest product (NTFP) [5, 7], are likely to have increased the spatial heterogeneity of populations. All species of the genus are listed in CITES [8], Appendix II, and several species are on the IUCN Red List [9]. *A. crassna* Pierre ex H. Lec. is classified as "Critically Endangered" [10] but with no field data attestation.

This is a case study on *A. crassna* and agarwood. Its first objective is to apply an inventory method to individual tree species occurring at low densities. Few methods exist for single species inventory, and only a small number of studies aim at single species *in situ* [11, 12]. The basic method used in this study is transect distance sampling. The second study objective is to assess the absolute density of *A. crassna* in harvested populations found within typical habitats at four sites in Northern Laos. This will help in facilitating resource assessment, reevaluating conservation status, and overcoming the deficiency of field data on the species.

Agarwood is a dark-coloured fragrant resin that accumulates inside stems and roots of *A. crassna* trees as nodules of varying age, shape, size, and commercial quality, embedded in the otherwise pale and soft wood. The production of this oleo-resin, which acts as a chemical barrier to attacks by fungi, insects, and so forth, is apparently a tree response to injury if its primary defence mechanism, the formation of

phloem callus tissue, is inhibited [13, 14]. The exact mechanism is not fully understood, and a number of ecological, physiological, and genetic factors may be involved. Not all trees form agarwood under natural conditions [4, 5].

Agarwood is traded under many synonyms, for example, eaglewood and *gaharu*, and in many different qualities of unprocessed wood and essential oil. It is used in medicinal, aromatic, ceremonial, and religious preparations and traded across old routes from its natural range States in Southeast Asia (Indochina and Thailand) to consumers in the Arab world and in East Asia [6, 15–17]. The greater part of agarwood comes from the wild [18].

The Lao People's Democratic Republic (henceforth Laos) was chosen as case study country because it is the only range State where harvesting of *A. crassna* is legal. Laos has been a major supplier of high-quality agarwood since harvesting started at the end of the Second Indochina War in 1975.

The taxonomic status of some *Aquilaria* species is uncertain. The information available from herbarium collections is scarce and access to the wild resources is difficult [17, 19]. In Laos, three different species were readily identified by interviewed harvesters based on tree or shrub habit, differences with regard to habitat, leaf and stem characteristics, and commercial quality of agarwood. About 80 to 90% of the agarwood traded is extracted from the species commonly referred to in Lao as *mai khedsana* and identified as *A. crassna* using Pham [20].

NTFP inventory approaches are often based on methods used in conventional forestry, that is, sample plots and design-unbiased sampling strategies. In scattered populations, these methods typically encounter only a few objects of interest and the point-to-point variability is high [21]. Assessment of the status of wildlife and bird populations is commonly based on distance sampling [22], and transect distance sampling is starting to be applied in forestry, for example, Safranyik and Linton [23].

2. Methods

2.1. Distance Sampling. Distance sampling is a method for assessment of the absolute density of a biological population, based on accurate distance measurements for all objects detected in the vicinity of a point or line [22]. Distance sampling appears to be useful when reliable estimates of density are needed for comparison between sites, through time or for the investigation of a single population. For objects that are sparsely distributed across large geographic areas, it is highly cost-efficient and often the only method available [22]. In line transect distance sampling, substantially more ground can be covered within a given time than in quadrate sampling, where all plants within quadrates must be counted if plant abundance is to be estimated without bias. Because plants often have a very patchy distribution, the ability to cover a large area of ground with modest resources is an important advantage of line transects. Furthermore, in line transect distance sampling, detection probability is modelled, whereas in quadrate sampling or strip transect sampling, detection is assumed to be certain, and although

this does not always hold true, the assumption is seldom tested [24].

The reason for the efficiency of the method is that the same number of detections is required to estimate density within large and small regions. In addition, estimation of absolute density is possible for a population even when only a relatively small percentage of the individuals, possibly as little as 10–30%, is detected within the area actually sampled, that is, within the maximum distance allowing individuals to be detected from the transect line. Furthermore, the population density estimate can be calculated from data collected by different observers, even if some observers fail to detect a lot of objects located at some distance from the line or point. Finally, it is not necessary to know the size of the region sampled [22].

Objects searched for were seedlings (height ≤ 0.5 m), saplings (height 0.5–2.0 m), standing trees (height ≥ 2.0 m), and felled trees of *A. crassna*. These objects are not equally conspicuous, and the probability of observing an individual located at a given distance from a transect lines therefore depends on object class. Thus, different detection functions apply, expressing the probability of detection as a function of distance. Perpendicular distances were measured from the transect line to each detected object of interest.

2.2. Survey Sites. Survey sites were identified using experienced harvesters' ethnobotanical knowledge, combined with information on historical patterns of harvest and trade. Harvested and unharvested populations are considered equally relevant, and both standing and felled trees are therefore sampled. The only requirement is that areas were first harvested within the past five to seven years, because the fast decomposition of the soft wood of *A. crassna* does not allow earlier utilisation to be tracked. The aim is to select sites that are typical of harvested populations in Northern Laos but may not necessarily be representative of these. Assessment of absolute density for the entire population in Northern Laos, or for the country as a whole, would require much larger inventory efforts and is therefore not aimed at.

At the survey sites, several subsites (habitats) were surveyed but because they were assessed to be ecologically similar and harvested in the same way, analysis and results are presented at the site level. Selection of survey sites was guided by ethnobotanical knowledge gathered as part of the study on—amongst others—characteristics of *A. crassna* populations and effects of agarwood harvesting. Sites were selected to represent populations harvested lightly and populations harvested repeatedly. A country-wide survey on harvest and trade of agarwood was conducted from February 2003 to March 2005. Semistructured interviews were conducted with harvesters ($n = 103$), agents, and traders ($n = 73$), and previously harvested areas and habitat characteristics were identified. This work formed the basis for identifying potential survey sites in typical *A. crassna* habitats with known harvesting records.

A presurvey was carried out in March and April 2004 with the help of two experienced agarwood harvesters, who also served as tree spotters during the line transect sampling.
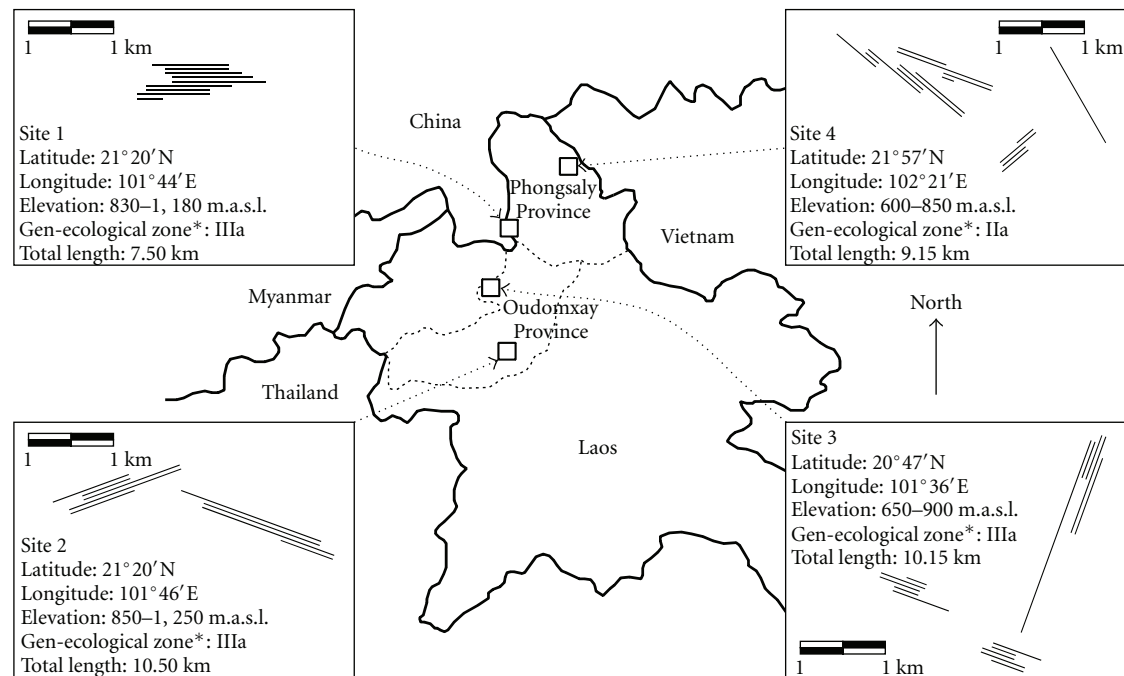
FIGURE 1: Location and description of survey sites. The four site maps indicate the location, length, and orientation of the track lines. *According to Greijmans and Inthavong [25]. IIa: mean temperature in coolest month, December <9°C, mean annual rainfall <2,000 mm and more than 3.5 dry months (mean monthly rainfall <40 mm). IIIa: mean temperature of coolest month, December 9°C–13°C, mean annual rainfall <2,000 mm and more than 3.5 dry months (mean monthly rainfall 40 mm).

Potential survey areas were visited, and it was examined if a total track line of approximately 10 km could be achieved. Distance sampling was carried out at four sites in Northern Laos in May 2004 and from January to March 2005. Two sites (1 and 4, cf. Figure 1) selected in Phongsaly Province were harvested for the first time in 2004 to supply an agarwood processing factory in Bountai District, and two sites (2 and 3) in Oudomxay Province had been harvested heavily and repeatedly from 2000 to 2002 to supply three factories in the provincial capital Oudomxay. At site 4, removal of wildlings had taken place. Heavy rainfall over weeks halted sampling at site 1, and only 7.5 km were therefore surveyed.

Agarwood harvesters engaged in the ethnobotanical survey identified A. crassna as an upper under-storey species occurring in patches throughout primary evergreen forests from 600 to 1,400 m above sea level. The natural distribution is largely confined to small mountains in areas next to streams and in the vicinity of rock outcrops. Harvesters further distinguished A. crassna habitats by their floristic composition, associated tree species, and stand structure. Soil type and soil characteristics seemed to be less important.

As mentioned, each survey area was composed of one or more subsites of A. crassna habitat. Details of the survey sites are shown in Figure 1. A total of 57 days were spent surveying transects, and 2,008 objects were detected along 37.3 km of transects. On average, 564 m of transects were walked per day. Although the sub-sites were similar with respect to basic ecological conditions and harvest intensity, differences in spatial extent are likely to imply that the within-site variation is greater for sites 2–4 than for site 1.

2.3. Survey Procedure. To achieve a total track length of 10 km at each survey site, track lines were established on 1–4 sub-sites (habitats) per site, see Figure 1. A sub-site consisted of parallel track lines of variable length with a distance between them of 50 m. Track lines consisted of track segments of 50 m each. To level out spatial variation, the track lines had a minimum length of 200 m (four track segments). However, in a few cases, and largely due to boulders, ravines, and steep slopes, shorter track lines were established. The starting point of the first track line was randomly chosen. At the end of the first track line, a parallel track line was randomly started, to the left or right. The parallel track lines were continued to the border of the A. crassna habitat. In case the A. crassna habitat was found on both sides of the first track line, parallel track lines were also laid out on the other side starting from the end of the first track line. The number of years since felling was estimated by visual inspection of the stage of decomposition of stumps and logging debris.

The survey team consisted of two experienced harvesters, acting as tree spotters, and a supervisor. Track segments were marked with a rope and tree spotters walked slowly on either side of the track segment. Standing individuals of A. crassna, that is, seedlings, saplings, and trees (henceforth: objects) were detected by their distinctive leaf characteristics: light-green, alternate, shiny but not reflecting sunshine, elliptic with a marked tip, and by their branches which bend 45 degrees upwards. Saplings and trees were further distinguished from other species by their smooth bark, often with spots, and their small open crown. Fallen leaves and

fruit further facilitated detection of standing trees. Felled trees were detected by the trunk, a stump of height 0.8–1 m with or without coppice, an opening in the canopy, debris, or, if the roots had been dug out, by a hole with a diameter of 2–5 m and a depth of 1-2 m.

When an object was detected, the perpendicular distance from rope to object was measured and recorded in metres to one decimal point. The trunk diameter at breast height (DBH, 1.3 m above ground) was measured using a diameter tape (accuracy 0.1 cm). For felled trees, DBH was measured on the lying trunk. If the stem had been removed, diameter was measured at the top of the stump, that is, often about 1 m above ground. If the roots had been dug out DBH could clearly not be measured.

2.4. Estimation of Absolute Density. Density was estimated using models in which distance to the object is the only covariate assumed to influence the detection probability, that is, Conventional Distance Sampling. The analysis was carried out using Distance 5.0 software [26].

The basic detection function $g()$ is expressed as

$$g(x) = k(x)[1 + s(x)], \tag{1}$$

where $g(x)$ expresses the probability of detecting an object of a particular type at the distance x from the track segment, $k(x)$ is a key function, and $s(x)$ is an expansion function increasing the overall flexibility of the detection function and thereby making it possible to adjust the function to a wider range of relationships between detection probability and distance from observer to object [22].

Based on the relative statistical fitness (using Akaike's Information Criterion) of various model alternatives and visual inspection of histograms showing the distribution of distances, it was decided to use two different key functions. A half-normal key function was used for saplings, trees, and stumps:

$$k(x) = \exp\left(\frac{-x^2}{2a^2}\right), \tag{2}$$

where a is a scale coefficient.

A hazard-rate key function was used for seedlings:

$$k(x) = 1 - \exp\left(-\left[\frac{x}{a}\right]^{-b}\right), \tag{3}$$

where a controls scale and b controls shape.

In both cases, cosine expansion functions were used

$$s(x) = \sum_{j=2}^{m} c_j \cos\left(\frac{j\pi x}{w}\right), \tag{4}$$

where $c_j (j = 2, \ldots, m)$ is model coefficients and w is width of the range within which trees were observed, that is, $x \in [0; w]$, the range being sufficiently truncated to remove obvious outliers from the material. In practice it was decided to truncate the seedling material at $x = 8$ m, whereas the distance observations for saplings, trees, and stumps were truncated at $x = 25$ m. With $j = 1$, the cosine function would

be monotonously decreasing with increasing x, and since this would not add to the flexibility of the overall model, $j \geq 2$ in (4). Moreover, c_j was in no case significant for $j > 3$ so in practice $m = 3$.

3. Results

The number of encounters, given in Table 1, is critically low for some sites, especially site 2, and object classes, especially felled trees. Density estimates for these must therefore be interpreted cautiously. The estimated mean encounter rates clearly indicate that the number of trees at sites 2 and 4 are considerably lower than at sites 1 and 3, whereas the number of seedlings, and saplings is particularly low at site 2 and the number of felled trees is considerably higher at site 4 than at other sites. The calculated dispersion indices are in most cases greater than 1.0 indicating that the spatial distribution of trees, seedlings and saplings is generally clustered, at least on the applied 50 m scale. Except for site 3, the dispersion index tends to decrease from seedlings over saplings to trees, indicating that larger individuals are more regularly distributed than smaller ones. Particularly high dispersion indices are observed for seedlings at sites 1 and 4 (31.7 and 30.8, resp.).

Details on recent exploitation, including percentages of felled trees with signs of agarwood formation and stumps with coppice, are shown in Table 2 and give an impression of harvesting impact. In addition, estimated mean diameters are included based on those of the felled trees for which DBH could be accurately measured. Besides depending on distance, detection probabilities are likely to depend on a number of factors, such as tree size, time since felling, and whether roots had been dug out. And the average size of trees for which DBH could not be accurately measured (e.g., excavated trees) may differ from that of other trees. Hence, generally valid mean diameter estimates cannot be provided. Attempts at including tree size in the detection function were not successful as some model parameters were highly correlated and convergence failed, presumably because the number of detections was too small. Since the effect of tree size on detection probability is not known, standard errors in Table 2 are estimated as for a simple random sample. Due to the fact that larger trees are less abundant than smaller individuals but can be detected at greater distance, the sample variance is presumably smaller than that of the population, implying that standard errors of the mean diameters are underestimated.

Site 4 had been harvested just before the survey took place. Site 1 was harvested in 2004 and in previous years. Sites 2 and 3 had been harvested prior to 2004, mostly in 2000–2002 it appeared. Approximately 69% and 38% of the detected felled trees had been dug out at sites 2 and 3, respectively. At sites 1 and 4, this practice had hardly taken place. The DBH of felled trees is likely to be larger than that of standing trees because the share of trees with agarwood is higher for large trees than for small. The large difference between estimated mean diameters of standing and felled trees is therefore not surprising. At site 2 the average DBH of

Table 1: Total number of detections (N), encounter rate (km^{-1}) plus/minus standard error, and index of dispersion: Var$(n)/\overline{n}$, where n is number of objects detected per 50 m transect.

Site		Seedlings	Saplings	Standing trees DBH < 10 cm	Standing trees DBH ≥ 10 cm	All standing trees	Felled trees
1	N	296	111	148	95	243	18
	Encounter rate (km^{-1})	39.5 ± 9.5	14.8 ± 3.9	19.7 ± 3.6	12.7 ± 4.9	32.4 ± 6.7	2.4 ± 0.6
	Dispersion index	31.7	4.1	2.1	3.7	3.6	1.6
2	N	54	48	118	6	124	36
	Encounter rate (km^{-1})	5.1 ± 1.8	4.6 ± 1.8	11.2 ± 1.3	0.6 ± 0.2	11.8 ± 1.3	3.4 ± 1.1
	Dispersion index	7.6	4.3	1.7	1.0	1.7	1.4
3	N	80	132	337	53	390	32
	Encounter rate (km^{-1})	7.9 ± 3.1	13.0 ± 2.6	33.2 ± 10.4	5.2 ± 1.2	38.4 ± 11.3	3.2 ± 1.2
	Dispersion index	7.6	3.9	21.3	5.0	25.0	1.7
4	N	159	135	135	13	148	65
	Encounter rate (km^{-1})	17.3 ± 10.4	14.7 ± 2.3	14.7 ± 2.5	1.4 ± 0.4	16.1 ± 2.6	7.1 ± 1.7
	Dispersion index	30.8	2.1	1.8	0.9	1.7	1.9
All	N	589	426	738	167	905	151

Table 2: Percentage of all detected felled trees with roots dug out, signs of agarwood formation, stumps with coppice, and felled in 2004 and before. Mean diameter of detected standing and felled trees. All figures plus/minus approximate standard errors. Felling year was not assessed for all felled trees.

Site	n	Per cent of felled trees detected ± Standard error					Diameter at breast height (cm)	
		With roots dug out	With signs of agarwood formation	Stumps with coppice	Felled in 2004	Felled prior to 2004	Standing trees (height ≥ 2 m)	Felled trees
1	18	5.6 ± 5.6	27.8 ± 10.9	44.4 ± 12.1	33.3 ± 11.4	44.4 ± 12.1	10.7 ± 0.5	32.1 ± 4.4
2	36	69.4 ± 7.8	83.3 ± 6.3	8.3 ± 4.7	5.6 ± 3.9	91.7 ± 4.7	3.6 ± 0.3	13.8 ± 3.2
3	32	37.5 ± 8.7	68.8 ± 8.3	31.3 ± 8.3	9.4 ± 5.2	90.6 ± 5.2	5.7 ± 0.2	31.4 ± 3.6
4	65	1.5 ± 1.5	12.3 ± 4.1	75.4 ± 5.4	96.9 ± 2.2	3.1 ± 2.2	4.5 ± 0.3	25.2 ± 1.5
All	151	25.8 ± 3.6	43.0 ± 4.0	46.4 ± 4.1	49.0 ± 4.1	47.7 ± 4.1	6.6 ± 0.2	25.6 ± 1.4

felled trees is very low because all the large trees had been dug out, and therefore the DBH could not be measured. About half of the stumps, 46%, had started to coppice, and the share of felled trees with signs of agarwood formation was 43%.

Coefficients of correlation between encounter rates calculated for seedlings, saplings, and standing and felled trees are shown in Table 3. In agreement with the clustering tendencies of seedlings and saplings indicated by the dispersion indices in Table 1, the correlations between encounter rates of such individuals are generally positive and statistically significant ($P < 0.1$). Encounter rates of seedlings and felled trees are generally significant ($P < 0.1$) and positive, indicating that clusters of seedlings are often found in gaps formed where trees have been felled. Similarly, encounter rates of saplings and felled trees are significantly positive except at site 4. As expected, encounter rates of standing trees with DBH > 10 cm correlate significantly with all standing trees.

Based on perpendicular distances between track segments and objects, the detection probability functions described in (1)–(4) were estimated for each site and object category. Examples for saplings and trees with DBH < 10 cm are shown in Figures 2 and 3, respectively. For both object categories the shape of the detection function varies little between sites. On the other hand, the graphs clearly indicate that trees are generally visible at greater distances than saplings, leading to a broader range around the transect line characterised by a high detection probability. Thus, for saplings the range within which the detection probability exceeds 0.5 is only 3-4 m, but for trees with DBH < 10 cm it is 8–10 m.

Based on the detection functions, effective strip widths (ESW) were calculated and are shown in Figure 4. ESW is calculated in such a way that the number of trees detected outside ESW equals the number missed inside it. Figure 4 shows that ESW increases with the size of detected objects: seedlings, saplings, and trees, but for felled trees the ESW is narrower than for standing trees. ESW varies between sites and is consistently most narrow at site 3 but the differences are not dramatic. Thus, it is likely that the sites are similar with regard to factors that influence detection, that is, vegetation structure and terrain.

Figure 5 shows estimates of densities per hectare for all six object classes, with 95% confidence intervals. Average densities in each of the six classes (thick lines) were obtained by pooling data from transects at all four sites. Unfortunately,

Table 3: Coefficients of correlation between number of detections per 50 m track segment for different categories of objects. *, **, and *** signify statistical significance at 0.1, 0.05, and 0.01 levels, respectively.

	Site	Saplings	Standing trees DBH < 10 cm	Standing trees DBH ≥ 10 cm	All standing trees	Felled trees
Seedlings	1	0.21***	0.23**	0.01[NS]	0.14[NS]	0.56***
	2	0.45***	0.10[NS]	−0.03[NS]	0.09[NS]	0.21**
	3	0.30***	0.07[NS]	0.08[NS]	0.07[NS]	0.20**
	4	0.16*	0.08[NS]	0.19**	0.12[NS]	0.16*
Saplings	1		0.21**	0.24**	0.27***	0.34***
	2		0.13[NS]	−0.04[NS]	0.12[NS]	0.39***
	3		0.34***	0.28***	0.34***	0.21**
	4		0.20**	0.16*	0.24**	0.10[NS]
Standing trees DBH ≥ 10 cm	1	0.24**	0.35***		0.83***	0.08[NS]
	2	−0.04[NS]	0.05[NS]		0.21**	−0.06[NS]
	3	0.28***	0.84***		0.88***	0.33***
	4	0.16*	−0.07[NS]		0.15*	−0.07[NS]

as the areas of the sub-sites (habitats) and the total areas of the sites are unknown, correctly weighted regional or national density estimates cannot be obtained. Hence, no confidence intervals are included for these broad averages. With the calculated averages, it is erroneously assumed that the total area of habitat at the four sites is proportional to total track length (see Figure 1). The average density estimates should be interpreted with this limitation in mind. A similar assumption of proportionality between area of sub-sites and their track length is used when estimating densities at the site level, but in this case the assumption is likely to hold as the sub-sites were sampled in their entirety.

Density estimates decrease consistently from seedlings to standing trees with DBH ≥ 10 cm and rise again for felled trees. Thus, as might be expected, the distributions of the preharvest populations are approximately reverse J-shaped. Standing trees are categorised in two diameter classes and, as expected, density estimates decrease as DBH increases. Trees with DBH < 10 cm have the same density as saplings, estimated at $10.6\,\text{ha}^{-1}$ and $10.9\,\text{ha}^{-1}$, respectively. The overall density of trees with DBH ≥ 10 cm, estimated at $1.7\,\text{ha}^{-1}$, is a key figure but it is important to note that the sitewise estimates vary from $0.2\,\text{ha}^{-1}$ to $4.4\,\text{ha}^{-1}$.

The overall estimate of the density of felled trees is $2.2\,\text{ha}^{-1}$, that is, greater than that of standing trees with DBH ≥ 10 cm ($1.7\,\text{ha}^{-1}$) and indicates that about half of the tree population at the four sites had been harvested.

For seedlings, the average density across all four sites was estimated at $39.8\,\text{ha}^{-1}$ (13.4–$73.5\,\text{ha}^{-1}$). This estimate is probably influenced by several factors. The survey year 2004 was a year without abundant fruiting so relatively few new seedlings presumably occurred. *A. crassna* has limited seed dispersal [27], and the digging out of roots has probably damaged or destroyed seedlings around mother trees. The collection of wildlings at site 4 did not appear to have any effect on overall density, probably because the collection was done by local people to fulfil an order of a fixed number of wildings, or because villagers did not find all seedlings.

Overall, harvesting has probably led to significant reductions in the number of standing trees and their average DBH.

Based on densities estimated for each of 48 sub-sites, it was attempted to relate the estimated densities of smaller classes to those of larger classes, for example, seedlings versus saplings, trees, and felled trees. However, no distinct patterns were revealed and regression analysis failed to detect any significant relationship. Thus, the impact of harvesting on the density of seedlings and saplings was only detected through the observed correlations between these objects and the felled trees (cf. Table 3).

4. Discussion

4.1. Survey Method. Based on the overall estimate of ESW, 12.9 m for standing trees with DBH ≥ 10 cm, and the total length of the transects at the four sites, 37.3 km, it is estimated that a total of 48 ha of *A. crassna* habitats was surveyed. Given that effectively 57 days were spent on transect walks, 0.65 km of transect corresponding to about 0.8 ha was covered per day. Hence, surveying the four sites using line transect sampling appears to be time and cost efficient but comparison with other methods should be considered in future studies. In the present case, sampling using ordinary nested quadrates or other types of fixed-area sample plots would have been an obvious alternative. But in all likelihood, the area covered per day would have been considerably smaller than 0.8 ha.

All four assumptions essential for reliable estimation of density [22] are likely to have been met. (1) The inanimate objects are all detected at their initial location; (2) a slow walking pace and careful instruction of tree spotters ensured that all objects located exactly on the track line were detected; (3) perpendicular distances were accurately measured and the measurements showed no indications of heaping. All measurements of perpendicular distances were made in the presence of the supervisor and frequently checked during the survey, and the tree spotters appeared sincere and
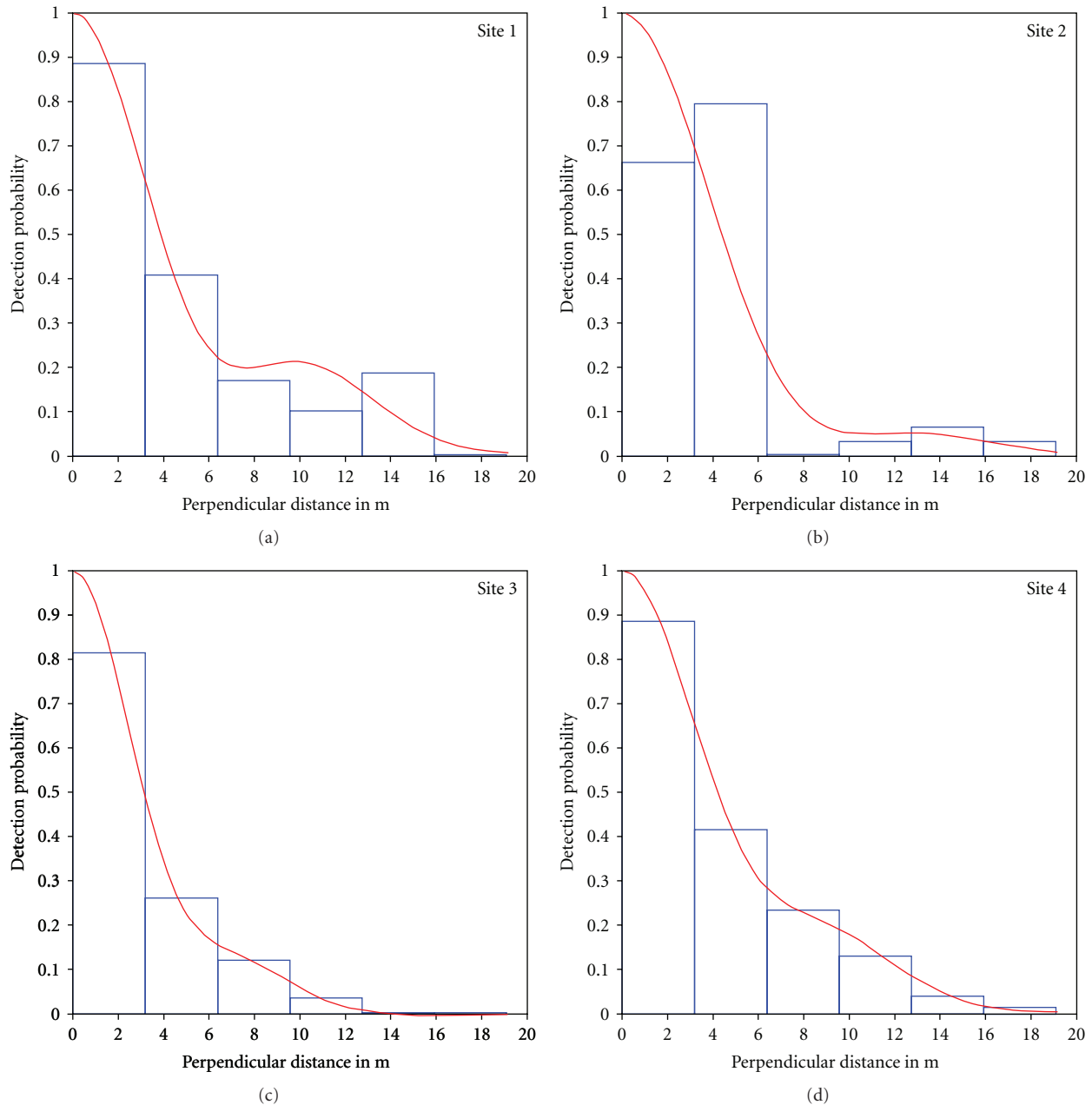
(a)

(b)

(c)

(d)

FIGURE 2: Detection probabilities for saplings at sites 1–4. Histograms represent the number of saplings observed at different distances from the transect line. Smooth curves are detection probabilities predicted by the detection functions. (Bar widths are selected automatically to match the range of distances and do therefore not match with tick marks).

enthusiastic throughout; (4) transects were randomly placed in the habitat with no bias towards certain habitat features or areas with high density, although tree spotters had some prior knowledge of the extent of populations from the presurvey.

Furthermore, all detection functions had a "shoulder," that is, the probability of detection remained close to 1 at short distances from the track line. This is not an assumption, but a property that allows reliable estimation of object density [28].

Buckland et al. [22] suggest that 60 to 80 detections are sufficient for robust and reliable estimation of a given species'

detection function. Unfortunately, the number of detections in this study is critically low for some objects and sites (Table 1). This problem is not easily solved if analysis remains at site level and a certain transect length per site is kept. Given the patchy occurrence of *A. crassna*, increasing the size of survey sites and transect lengths is often not possible. At the analysis stage, detections from two or more sites could be merged if utilisation patterns and ecological zone are deemed similar. To produce reliable estimates, this would require that the detection functions are similar and that the area of habitats at each survey site is known, thereby enabling correct weighting of density estimates. Alternatively, if sites
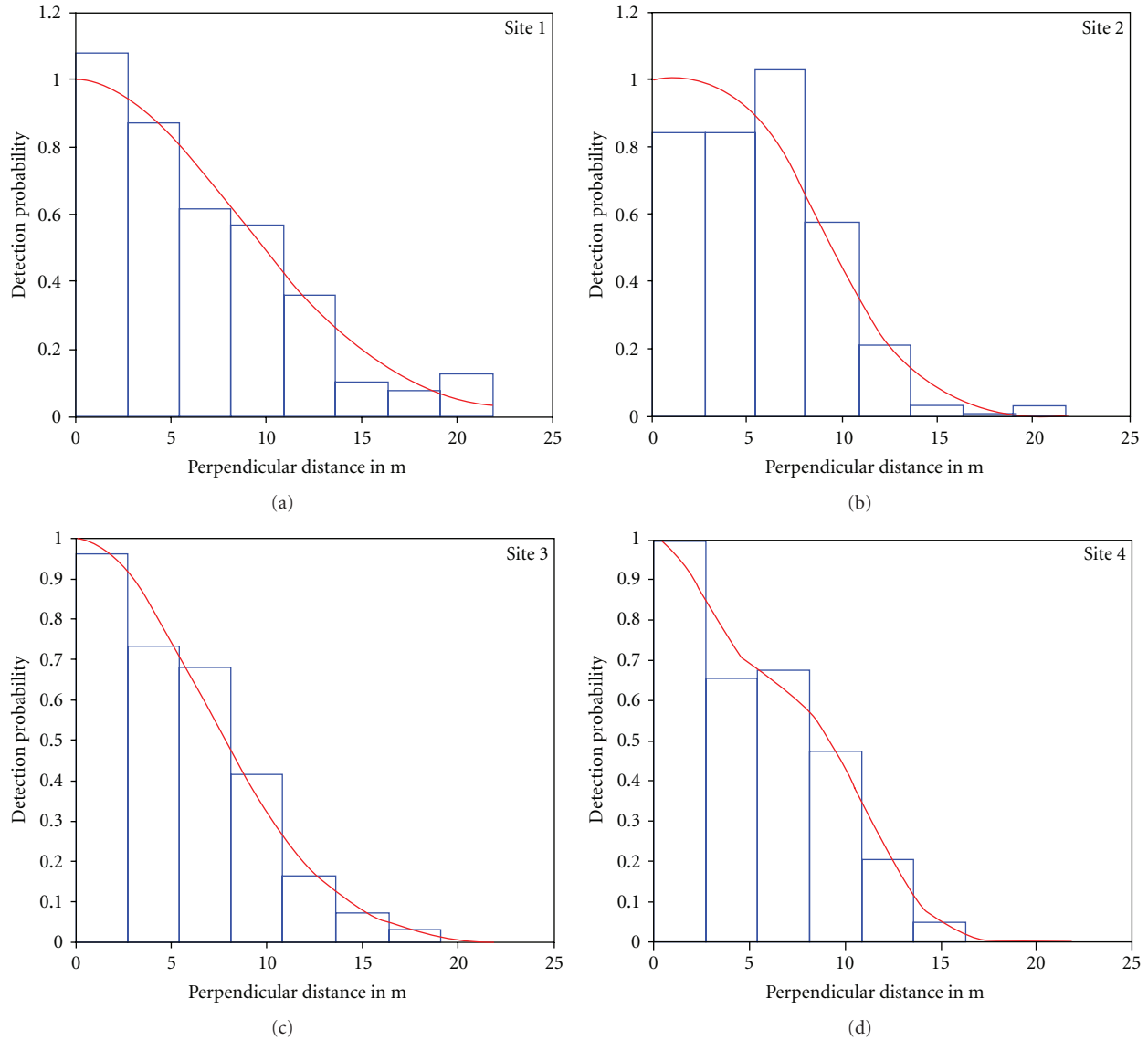
(a)

(b)

(c)

(d)

FIGURE 3: Detection probabilities for trees with DBH < 10 cm at sites 1–4. Histograms represent the number of trees observed at different distances from the transect line. Smooth curves are detection probabilities predicted by the detection functions. (Bars are scaled such that their total area is equal to the area under the detection probability function (red). Bars may therefore exceed 1, as seen for site 1).

are expected to differ with respect to the detection function, density estimation could be done using Multiple Covariates Distance Sampling (MCDS) with site or other variables as covariates, explaining the differences between sites. In the present case, such approaches were tried but frequently failed to produce results, leading to the choice of Conventional Distance Sampling.

An alternative inventory method is adaptive cluster sampling (ACS) which has been suggested for rare and clustered populations [29]. It has been applied to rare species in Nepal [30] and to *Prunus africana* in Cameroon [11]. With prior knowledge of typical *A. crassna* habitats from two complementary sources, that is, a trade survey and an ethnobotanical survey, and given the potential difficulties in establishing decision rules for ACS, distance sampling was preferred to ACS. However, distance sampling is not

applicable to very rare species because of the number of detections required for the statistical analysis. In such cases ACS may prove more flexible, provided that the species is clustered, and also offers the option of collecting additional ecological data within the clusters.

4.2. Population Status. Data for this study were collected in 2004 and 2005. Based on interviews with experienced harvesters and the nationwide survey, it is believed that no harvesting has taken place in the area since then, and results should therefore also apply to 2011 (with adjustment for growth and population dynamics). The IUCN categorisation of *A. crassna* is mainly pertinent to Vietnam [10] and reflects a 90% reduction (IUCN 2006) in the number of mature, reproductive trees due to heavy exploitation and habitat loss.
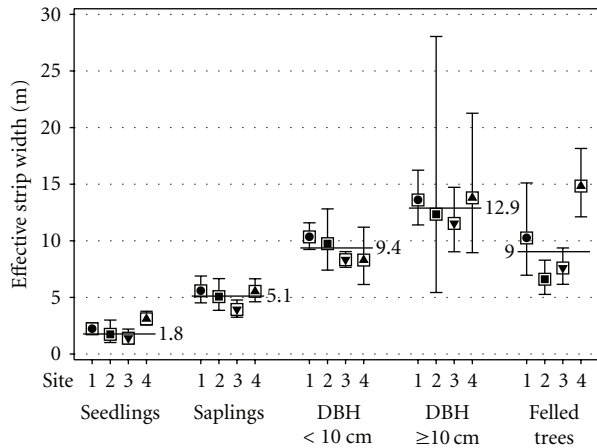
FIGURE 4: Effective strip width (ESW) with 95% confidence intervals for various object classes. Thick horizontal lines with numbers indicate the average ESW for each category across the four sites. Separate symbols are used for each of the four sites.
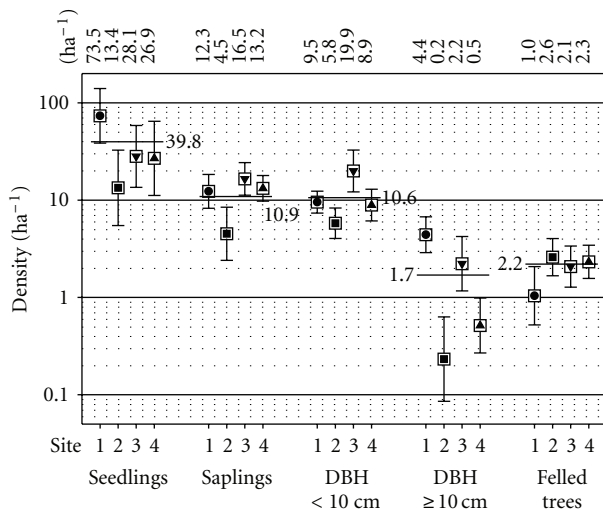

FIGURE 5: Density estimates for various object classes with 95% confidence intervals. Thick horizontal lines with numbers indicate average for each category across the four sites. Separate symbols are used for each of the four sites. Note: logarithmic scale on ordinate axis.

If it is assumed that very little harvesting took place prior to 1998, the overall reduction of the density of standing trees with DBH $\geq$ 10 cm at the four sites investigated in this study seems to be c. 60%. Thus, the situation at the four sites in Laos cannot currently be said to be similar to that described for Vietnam.

By adding up the density estimates of felled and standing trees with DBH $\geq$ 10 cm, the density of the preharvest population can be estimated. The overall estimate of 3.9 trees ha^{-1} indicates that A. crassna is a relatively common tree species within the habitat areas surveyed. Absolute density estimates from this study may not be comparable with estimates from other studies (e.g., [3–5, 7]), because it is unclear whether these estimates are general or specific to habitats of Aquilaria spp. as in this study.

Site 2 deviated markedly from the other three sites with respect to the estimated densities of standing trees, saplings, and seedlings (cf. Figure 5). This may be a consequence of factors not related to human exploitation. Hence, future studies should include effects of altitude and subforest type.

It is unlikely that commercial timber harvesting is taking place in A. crassna habitats. They are located in rather inaccessible mountain regions, and generally the species is associated with less valuable timber species. If any conversion to other land uses is taking place, it is related to shifting cultivation, and of limited effect since repeated burning is mainly done close to human settlements and, in most cases, far away from A. crassna habitats. The current deforestation at the rate of 0.79% per year [31] is therefore not expected to have affected A. crassna habitats.

Evergreen forest is a dominant forest type in Laos [32] but the distribution of A. crassna is not documented. No sources of data permit estimation of total area with A. crassna habitats as the species was not included in the nationwide forest inventory at species level conducted in Laos 1992–1998 [33]. At that time A. crassna was not considered an economically important species by the Lao Department of Forestry in charge of inventory design and implementation.

Although the felling of large trees had been extensive at the four sites surveyed, evidence from this study suggests that harvesting is not likely to cause extinction of A. crassna. Saplings and small trees do not seem to be the object of harvesters. There were also indications of active regeneration in the form of seedling growth and possibly regrowth following felling.

Estimated densities of trees, both with DBH $\geq$ 10 cm and in the smaller object classes, did not indicate a species in decline at the four sites, although harvesting is likely to have resulted in a reduced population mean diameter.

The extent to which these populations will recover from harvesting, assuming that harvesting takes place at long intervals, depends on recruitment rates and juvenile mortality. In plantations it has been observed that flowering and fruiting starts at an early age, and early fruiting is also observed at forest fringes around gaps newly opened up by shifting cultivation. Soehartono and Newton [27] report the flowering of a tree with DBH 12.7 cm. Indeed, also during this study flowering of small trees was observed at site 1. Even with low densities, continued reproduction may be possible because Aquilaria is monoecious, and there is no published evidence of self-incompatibility [5].

Canopy gaps formed by removal of large mother trees may result in conditions similar to those in plantations, possibly promoting abundant seed production in young trees and perhaps even in coppice, and leading to higher survival rates of seedlings and saplings. Paoli et al. [5] reported that seedling survival was positively correlated with distance from the mother tree, a correlation that may to some extent be related to light conditions. Gunn et al. [34] note a higher incidence of saplings in harvested areas.

Large fruit-producing trees often show signs of agarwood formation. Conversely, large trees left behind after repeated harvesting may not have formed agarwood in commercial quantities. Since agarwood formation may be linked to

a genetic disposition, seed collection in harvested natural forests may not be ideal as seeds could potentially originate from genetically inferior trees.

Harvesting has resulted in commercial extinction in a large proportion of the habitat areas and this limits rural livelihood options linked to sustainable management of *A. crassna*. Species management should focus on (1) ensuring long-term commercial viability through managed harvest, (2) minimising losses by only harvesting trees that have actually formed agarwood, and (3) leaving behind a sufficient number of good quality mother trees.

Acknowledgments

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