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Comparison of visual observation and excavation to quantify density of the endangered bivalve *Unio crassus* in rivers of north-eastern France

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ABSTRACT

Key-words: *unionid,* Unio crassus*, threatened species, sampling design, population structure*

In the context of a rapid decline in several protected unionid species, government agencies urgently require a reliable method to estimate population size for the most endangered species. We used a dataset collected from 16 river stations in north-eastern France to compare the efficiency of visual estimation (bathyscope) and a manual excavation to estimate numbers of the endangered bivalve *Unio crassus*. Our investigations indicated that, whereas a visual approach was sufficient to detect unionid presence, only 10% of all individuals were registered compared with manual excavation at the same site. In order to obtain an accurate density estimate (especially as regards the juvenile population), sediment excavation is necessary, despite it being time consuming and damaging to the mussel's habitat.

RÉSUMÉ

Comparaison de densités obtenues par excavation et par observation visuelle du bivalve menacé *Unio crassus* dans des rivières du Nord-Est de la France

Mots-clés : *unionidae,* Unio crassus*, espèce menacée, plan d'échantillonnage, structure de population*

Dans le contexte d'un déclin rapide de plusieurs espèces protégées de bivalves unionidés, les organismes en charge de la gestion des milieux aquatiques ont un besoin urgent d'une méthode fiable pour estimer la taille des populations des espèces les plus menacées. Nous avons utilisé un ensemble de données recueillies sur 16 stations de différentes rivières du Nord-Est de la France pour comparer des estimations visuelles par bathyscope et des comptages réalisés en excavant le substrat dans le but d'estimer des densités d'*Unio crassus*. Nos résultats indiquent que si une approche visuelle est suffisante pour détecter la présence de cette espèce, seulement 10 % en moyenne des individus excavés ont été dénombrés visuellement. Il en résulte que pour obtenir des valeurs précises de densités (et de la proportion d'individus juvéniles), l'excavation du sédiment est nécessaire, bien que cette méthode soit chronophage et impacte l'habitat d'*Unio crassus*.

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INTRODUCTION

The thick-shelled river mussel *Unio crassus* (Philipsson, 1788) is a declining species listed in Annexes II and IV of the European Habitats Directive (Council Directive 92/43/EEC) and as Endangered by the IUCN (Van Damme, 2011). *Unio crassus* has been assessed on national Red Lists as Critically Endangered in Germany, Switzerland and Austria, vulnerable in Albania and extinct in the Netherlands (Van Damme, 2011). Further research is urgently required to assess distribution and population trends in order to set up active site protection and help reverse population declines.

Knowledge and protection of rare species requires an accurate estimate of their density and population structure. Three main methods are commonly used to inventory freshwater bivalve populations: visual estimation, manual search and excavation of individuals (Strayer and Smith, 2003). Unionid mussels are sedentary benthic invertebrates that feed primarily through water filtration. For that purpose, they must open their valves and empty their siphons into the water column. They can be observed relatively easily during this activity despite their cryptic shell coloration and shape.

Our aim was to compare two methodologies (visual approach *vs*. excavation) used to quantitatively estimate *U. crassus* density and population structure (*i.e*. juvenile *vs.* adult individuals) based on data collected from 16 river stations in north-eastern France.

MATERIAL AND METHODS

> *STUDY SITES*

Sixteen study locations were chosen from across northeastern France where presence of *U. crassus* was known (Table I). The exact location of the starting point was randomly selected and we explored 50 m^2 of the river bottom, across the river's width. All surveys were undertaken in June when water levels were lowest. The greatest possible diversity of river types was sought during the selection of river stretches. A short description of the 16 locations is also provided in Table I.

> *QUANTIFICATION OF UNIO CRASSUS POPULATIONS*

Visual counts of *U. crassus* individuals were first undertaken over a 50 m^2 quadrat (bank-to-bank) using a bathyscope. The bathyscope was used systematically in order to avoid reflections and the effects of image distortion on the river bottom. Photographs of colleagues using the bathyscope to observe *U. crassus* can be found in Zettler and Jueg (2007). During the visual search, one person was placed with a bathyscope every meter along the river's width and only living individuals were counted. All participants progressed side by side and observed the entire substrate bottom of the station. Calculated search speed ranged between 0.9 $m^2 \cdot min^{-1}$ and 6.2 $m^2 \cdot min^{-1}$, with an average of 3.2 $m^2 \cdot min^{-1}$.

Subsequently, 20×0.25 m^2 quadrats were randomly selected from the same 50 m^2 sample area. The 0.25 m^2 sampling unit has frequently been used by malacologists in the past (*e.g*. Miller and Payne, 1992; Vaughn *et al.*, 1997; Aldridge, 2000; Smith *et al.*, 2001; Pooler and Smith, 2005; Piette, 2005). Further, Strayer and Smith (2003), when comparing mussel densities estimated from different sized quadrats (*i.e*. 0.25, 0.50, 1, 2 and 4 m^2), noted the smallest variation coefficient with quadrats of 0.25 m^2. Each 0.25 m^2 quadrat was subjected to visual exploration using a bathyscope, following which the sediment of the same quadrat was excavated to a depth of 10 cm (see Miller and Payne, 1992; Vaughn *et al.*, 1997; Smith *et al.*, 2000). In this way, an area of 5 m^2 (*i.e*. 20×0.25 m^2), or 10% of the 50 m^2 sample area, was subjected to double sampling. According to Smith *et al.* (2000) and Strayer and Smith (2003), excavation appears to damage both the river substrate and the bivalves themselves. Indeed,

Table I
Location of the 16 study sites, with a summary of the site characteristics. Uc = Unio crassus, Up = Unio pictorum, Aa = Anodonta anatina.

River	Locality	*X*	*Y*	Strahler Rank	Surface (km^2)	Altitude (m)	Slope (%)	Width (m)	*Uc*	*Up*	*Aa*
Aire	Rarécourt	854 856.308	6 888 253.72	3	345.0	201	0.138	5.1	X		
Armance	Avreuil les Bores	775 089.110	6 772 946.36	3	170.5	125	0.092	6	X	X	X
Bar	Laneuville-á-Maire	832 976.667	6 943 436.60	3	276.9	162	0.021	5	X		X
Brenon	Ogneville	925 324.245	6 821 567.13	3	60.7	273	0.500	4	X		X
Esch	Gézoncourt	919 914.475	6 863 544.66	3	180.0	214	0.135	6	X		
Halbmuhlbach 1	Biblisheim	1 050 916.700	6 876 707.53	3	11.0	143	0.129	5	X		X
Halbmuhlbach 2	Walbourg	1 055 395.570	6 875 517.21	3	11.0	143	0.129	4.1	X		X
Longeau	Harville	899 442.827	6 891 962.74	3	121.0	200	0.133	4	X		
Longeau	Friauville	908 320.376	6 897 624.02	3	157.2	193	0.085	6	X		X
Longeau	Brainville	906 174.065	6 896 922.41	3	157.2	193	0.085	4	X	X	X
Mazeley	Mazeley	950 349.056	6 800 628.47	3	74.3	316	0.221	4.1	X		
Meuse	Bannoncourt	883 401.270	6 876 059.16	5	2964.0	205	0.039	21.5	X	X	
Ornain	Alliancelles	838 542.287	6 857 180.42	3	893.1	137	0.136	8	X	X	
Sanon	Parroy	966 205.429	6 848 269.60	4	128.5	226	0.106	6	X		X
Sormonne	Chilly	806 317.489	6 971 568.00	3	75.1	209	0.174	4	X		
Voire	Challette/Voire	805 632.840	6 816 875.14	4	914.2	111	0.041	12	X	X	

this sampling method can lead to higher bivalve mortality, reduced rate of growth and interference in reproduction. For these reasons, we limited our sampling effort to just 20 quadrats and excavation of 5 m^2 of substratum.

The sediment was then passed through a 6.14 mm mesh sieve in order to recover both adult and juvenile individuals. For analysis, we considered all individuals measuring less than 30 mm (longest shell length) as juveniles and all individuals of more than 30 mm as mature individuals. Only those Unionids with no fouling on the shell were assessed.

RESULTS

The density of *U. crassus* excavated per quadrat ranged between 0.44 (Halbmuhlbach 2) and 66 (Esch) ind$\cdot$m^{-2}, with an average of 6.36 ind$\cdot$m^{-2}. Juveniles were only observed during sediment excavation and sieving (*i.e*. not during visual observations), with a total of 36 juveniles (length $<$ 30 mm) observed from seven sampling quadrats on five rivers (3 quadrats at Longeau, and 1 quadrat each at Esch, Armance, Ornain and Sormonne). The smallest individual found was 12 mm long and was found in the River Esch. The number of individuals excavated from stations where juveniles were not observed ranged between 2 and 11 (for 20×5 m^2 quadrats), and between 7 and 165 in the others. Where observed, juveniles represented between 9.4 and 25% of all individuals excavated.

The ratio between visual counting over the whole 50 m^2 survey area and visual counting from 20 quadrats was 6.1 $\pm$ 4.06 (mean $\pm$ standard deviation). While the proportion of individuals observed at the station level was highly variable (variation coefficient = 66.5%) compared with that observed in quadrats, the mean ratio was relatively stable, depending on the density observed (Figure 1). The ratio between visual counting over the whole 50 m^2 survey area and excavation of 20×0.25 m^2 quadrats was 12.47 $\pm$ 6.1; meaning that for every 10 individuals excavated from the river bottom an average of one was observed with the bathyscope at the station level (*i.e*. 10%). Note that these were exclusively mature ($>$30 mm) individuals. Again, the proportion of individuals observed at station level was highly variable (variation coefficient = 49%) compared with that excavated from quadrats, though the mean ratio was relatively stable depending on the density observed (Figure 1).

DISCUSSION

Future protection of *U. crassus* requires a simple, fast and reliable method for estimating population size at a given location. A comparison of the advantages and disadvantages of the various methods available for sampling Unionids (Table II) would suggest that visual observation (using a bathyscope) represents a good compromise method. To be applied on a large scale by environmental agencies, however, the efficiency of the method must be known Further, in addition to being simple and cheap to use, the method must also ensure that several operators working independently at a given location will obtain the same range of values. Our results suggest that visual observation is a method that allows a good rough estimation of *U crassus* populations, with the facultative option of excavation to evaluate population structure more precisely. Both methods are convenient and can potentially be applied easily.

Based on our results, it was possible to identify to species level using the visual method without disturbing the mussels, though this is clearly not quantitative. The difference between abundances observed in quadrats visually and that obtained from sediment excavation (a constant ratio of 1 to 10 in our study) may be due to both the mussel's cryptic efficiency and it's burying behaviour (Vaughn *et al.*, 1997; Smith *et al.*, 2000) as only epibenthic mussels or those not fully buried in the sediment will be found during sampling restricted to the substrate's surface (Amyot and Downing, 1991). While we cannot exclude that brightness, water turbidity and water depth may also have impacted on visibility, we could not test for these factors during this study. In addition, visual search results could also depend on factors such as the visual acuity and fatigue of observers (Strayer *et al.*, 1997) or their ability to observe

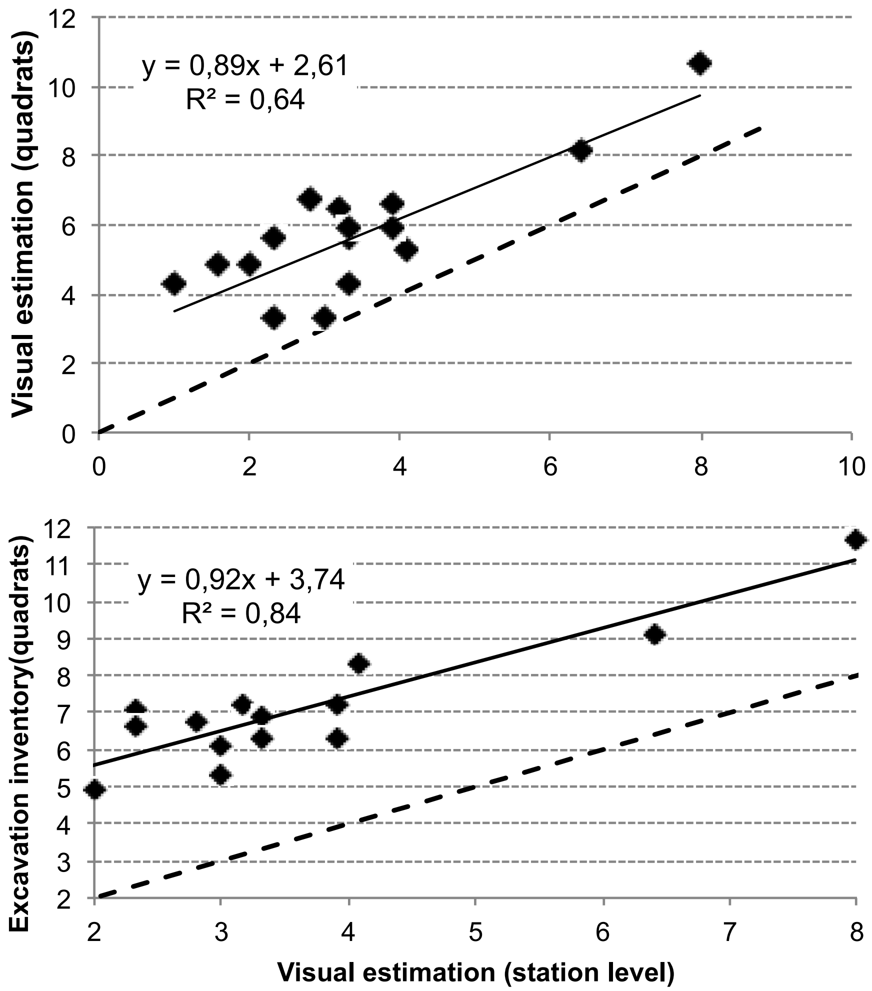

Figure 1

Comparison of Unio crassus abundance ($\log_2$ *(abundance)) estimated using two different approaches (n = 16 locations). Upper: U. crassus count in 20 × 5 m*^{2} *quadrats (within a 50 m*^{2} *stretch) extrapolated to 50 m*^{2} *vs. visual counting over the same 50 m*^{2}*. Lower: U. crassus count in 20 excavated 5 m*^{2} *quadrats extrapolated to 50 m*^{2} *vs. visual counting in the same quadrats. The dotted line represents an equal number of individuals observed using either method (y = x). Regression analysis was calculated on* $\log_2$ *transformed data.*

mussels over a large area during the study. This may well account for the observed ratio of 1 to 6 observed between visual counts at the station level and those using quadrats.

In order to obtain more quantitative population data some degree of sediment excavation and sieving will be required. While this approach is both time consuming (from 0.5 to 10 $m^2 \cdot h^{-1}$ according to Smith *et al.* 2000 and our own observations) and expensive (Obermeyer, 1998), it does allow for evaluation of juvenile density, vitally important if a declining population is suspected. Distinction between adults and juveniles is important as low juvenile densities indicate reproduction and recruitment failure, and aging populations are more susceptible to threats (Bauer *et al.*, 1991). In our study, no juveniles were observed during visual surveys, further supporting the need for some degree of excavation analysis. While our result of 10% of all individuals observed through visual observation alone allows for a rough estimate of actual density, some degree of sediment excavation will still be needed to obtain a more precise evaluation of *U. crassus* population structure.

Table II
Comparison of positive and negative features of the various sampling methods available for Unionid surveys.

Methods		Positive	Negative
Visual	**During diving**	- High rate of exploration - Allows coverage of a large area - Qualitative data - Usable in almost all streams	- Requires specialist equipment and training - Safety
	Bathyscope	- High rate of exploration - Allows coverage of a large area - Qualitative data - Usable in wadeable streams	- Impossible if water levels exceed 1.2 m - Does not allow a quantitative approach - Strongly and completely buried individuals are not visible
Tactile			- Impossible if water levels exceed 0.6 m - Efficiency depends on substrate type - Health risk and risk of injury - Time consuming
Excavation		- Essential for recruitment estimation - Coupled with a sampling model it allows a quantitative approach	- Low rate of exploration (time consuming) - Does not allow coverage of a large area - Disturbs the ecosystem

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