Research Article

Ecology and conservation of the endangered Indochinese freshwater pearl mussel, *Margaritifera laosensis* (Lea, 1863) in the Nam Pe and Nam Long rivers, Northern Laos

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Abstract

In this paper we present the first ecological data of Indochinese freshwater pearl mussel, *Margaritifera laosensis* populations. We also provide a comparative study of the ecology of this tropical species with populations of other Margaritiferidae. We conducted surveys in ten tributaries of the River Nam Ou (Middle Mekong Drainage, Northern Laos). Reproductively viable populations were found only in the Nam Long and Nam Pe rivers, which are two of the only three known viable populations of this species in the world. The habitats of *M. laosensis* include mountainous oligotrophic rivers with circumneutral pH. Optimal mesohabitats are riffles and runs with a median depth of 0.2 m and median current velocity of 0.3 ms^{-1}. Pearl mussels were more common in gravel and fine gravel riverbed substrates. Surveys revealed 252 specimens, but only 78 (31.0%) were alive. The largest mussels observed were 110 mm in length and only 11-12 years of age. The presence of smaller-sized mussels indicates recent recruitment in both populations. The most significant threats to *M. laosensis* populations are harvest by local people and land development in the River Nam Ou Basin.

Keywords: Margaritiferidae, Mekong River, tropical river headwater, deforestation, artisanal harvest

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Introduction

The freshwater pearl mussel family Margaritiferidae includes 13 species in the genera *Margaritifera* and *Cumberlandia* that are mainly distributed in temperate latitudes of the Northern hemisphere [1, 2]. Only *M. laosensis* inhabits tropical river systems [3]. To date, this species has been under studied and few fundamental ecological traits have been documented.

The type locality of *M. laosensis* is located within the "Laos Mountains, Cambodia, Siam" (Lea, 1863). Lea had only two specimens for description and his vague type locality description may reflect the typically haphazard collecting notes of the day as well as the region's unclear political boundaries at the time. The area covered by Lea's type locality description includes portions of present-day Laos, Cambodia and Thailand [4]. According to several historical reports, *M. laosensis* occurred in the Salween and Mekong river drainages in Northern Burma (Myanmar), Northern Laos, Northern Thailand and Northwestern Vietnam [1, 3-5]. In Myanmar, *M. laosensis* has been collected in the Karin Hills [6], including streams near Lake Inle [7]. Two specimens from the Inle Lake area (Fort Stedman, Shan States) were described as a separate species, *Margaritifera woodthorpi* [7], that is now considered a synonym of *M. laosensis* [3-5]. Recently, *M.laosensis* was reported from two tributaries of the River Nam Ou, the largest tributary of the Middle Mekong in Northern Laos [8, 9].

In Vietnam, *M. laosensis* are reported from streams near the city of Dien Bien Phu, Nam Ou Drainage [10] and in a tributary of the Ubolratana Reservoir, Northeastern Thailand [11]. Brandt [4] found subfossil shells in the River Pai (Salween Drainage) in Mae Hong Son Province, Northwestern Thailand (at an archeological site). Marwick and Gagan [12] conducted intensive quantitative sieve-based sampling over several river km in Northwestern Thailand (Pai Basin), but did not locate live specimens or shells of *M. laosensis*.

Most basic attributes of *M. laosensis* biology, ecology and habitat preferences are unknown, as well as what factors are major threats to Indochinese pearl mussels in this rural part of their range. The Indochinese pearl mussel is of considerable interest because it is the most southern-occurring species in the family Margaritiferidae and because it is presumed to be rare and declining [3, 10]. The majority of stressors to pearl mussel populations originate from economic activity, including the construction of dams and flow regulation [13-18]; road building [19]; felling and ditching of forests [17, 19]; mining [20]; and from urbanization and agriculture [13, 21, 22].The present study represents the first description of the habitat and ecology of *M. laosensis*. In addition, we describe the major threats and recommended actions for *M. laosensis* conservation.

Methods

We conducted field surveys in 10 tributaries of the River Nam Ou, Phongsali Province, Northern Laos, in 2012 (Fig. 1).

Annual mean temperature in Phongsali is 19.8 ºC and annual rainfall is 1,511 mm [23]. Due to monsoonal atmospheric circulation patterns, the Nam Ou Basin is characterized by alternating rainy and dry seasons. Precipitation during summer monsoons results in widespread flooding, and during dry seasons stream discharges are much lower [23]. Approximately 80% of annual runoff occurs during the summer monsoon (June-October). Many Northern Laos summer river levels exceed winter base-flow levels by 1.5–2.0 m, and discharge during wet seasons may increase 5–6x [23].

The Nam Long River is 29 km long, drains a 282 km^2 catchment, and drops 340 m between its source and mouth. The Nam Pe River is 36 km, drains a 234 km^2 catchment, and falls 510 m between its source and mouth. Both rivers' valleys are highly incised with a V–shaped cross-sectional profile. The slopes of river valleys are covered with evergreen highland tropical monsoon forest, and riverbanks are dominated by shrubs and bamboo thickets (Fig. 2A). Maximum river depth ranges from 2–3 m in pools and 0.1-1.5 m in rapids. Riverbeds are characterized by numerous hard rock outcrops (clay-rich slates), with tectonic faults that form stepped waterfalls (2–3 m total height) and stretches of very deep (> 3 m) water. The geology of many river basins inhabited by *M. laosensis* is characterized by clay-rich slates and continental red clay sandstones with occasional thin coal seams and conglomerates [23; M. Gofarov , pers. com.].

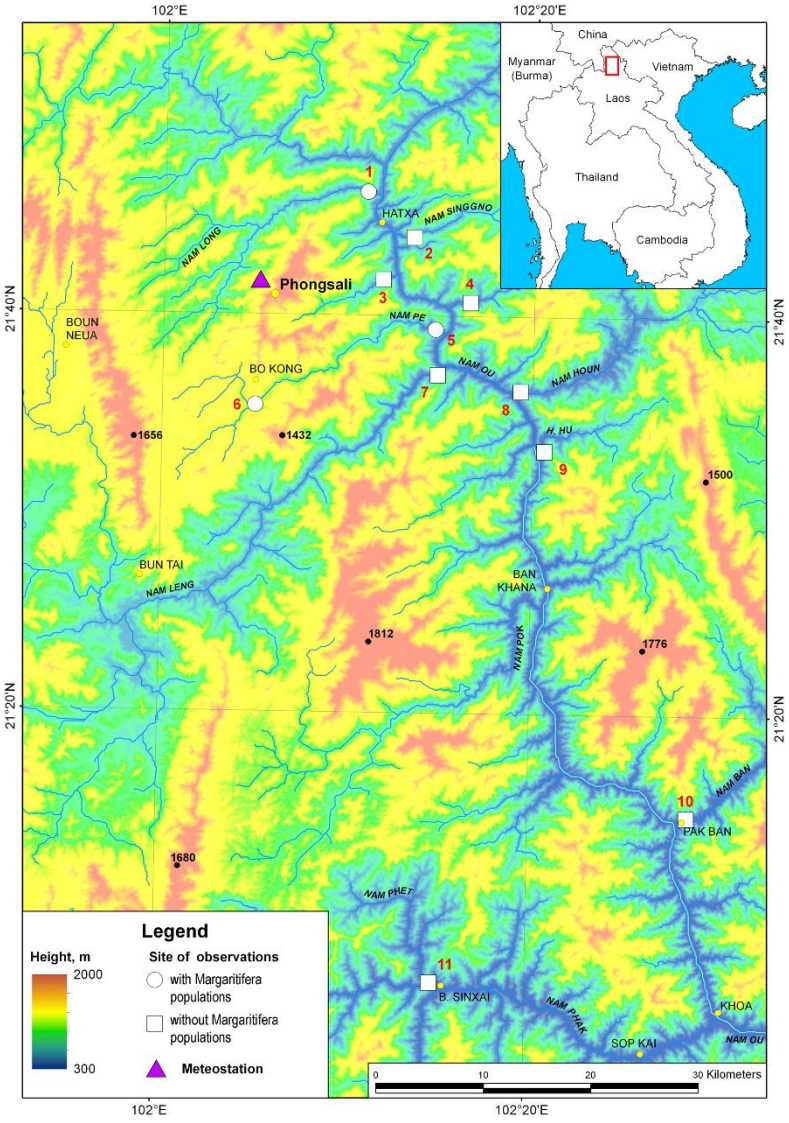

Fig. 1. Map of the Indochinese Peninsula with boundaries of study area (in insert) and map of the Upper Nam Ou River drainage with surveyed sites: 1 – Nam Long River; 2 – Nam Singgno Stream; 3 – Nam Phan Stream; 4 – Nam Ong Stream; 5–6 – Nam Pe River (5 – downstream, 6 – upstream); 7 – Nam Leng River; 8 – Nam Houn River; 9 – Nam H. Hu Stream; 10 – Nam Ban River; 11 – Nam Phet Stream.

All surveys were conducted during low water levels using a mask and snorkel to examine substrates and overturn large rocks to find *M. laosensis* [24]. Live mussels (Fig. 2B) were measured with calipers (maximum shell length) and returned to the river at the site of capture to minimize disturbance to mussels [25]. Empty shells were collected and retained. Intact shells collected from sand and plant litter on the river shores were considered to have died from natural causes; the majority of these shells were found in the Nam Long River. In addition, we sampled fresh-dead valves with remains of soft tissue that had been harvested from the Nam Pe River by local villagers; these shells have traces of cutting by knives or splitting by stones (Fig. 2C). Empty shells were measured with calipers to assess which age classes are most vulnerable to artisanal harvesting and natural mortality.

Fig. 2. *Margaritifera laosensis*: A – one from observation sites in the river Nam Long (riffle; NL3); B – living specimen in the river Nam Pe; C – shells harvested by local people, downstream of the river Nam Pe at the confluence with the river Nam Ou.

Mussel ages were estimated by counting the growth lines (annuli). Shell preparation for growth line counting was followed to standard method [28-30]. We hypothesized that the growth line of *M. laosensis* is formed in winter, and yearly individual growth can be estimated from length between consecutive growth lines because the growth lines of other margaritefirids, especially those that also have a southern range (*M. laevis*, *M. auricularia*, *M. falcata* and *M. hembeli*) are known to form in winter [31-34]. Growth rings of *M. laosensis*, in comparison to *M. margaritifera*, for example, are wider and clearer, and therefore easier to count. Estimates were

made for the three smallest and three largest individuals from each river (n = 12). Annual shell rings provide an approximate age, but rarely reveal early growth because the anterior dorsal part of the shell (umbo) is frequently eroded. We measured the eroded part of shells and used the number of annuli on the youngest (four years old) mussels found to estimate total age.

We quantified habitat conditions in different mesohabitats. Mesohabitat types were assigned following the criteria of Frissell et al. [26]. Riverbed substrate types were estimated using the classification system of Platts et al. [27].

River depths were measured using a ruler. Drifting time was fixed by stopwatch and then calculated to m/s. Water temperature was measured in the bottom layers using a digital thermometer TK–5.05 ("TECHNO-AC", Russia). Ten water samples were collected across all sites for water chemistry analysis in 0.5 l plastic bottles. Samples were cooled immediately to 2–4 °C and stored in the dark at the same temperature until analysis. pH values were measured by the potentiometer method using a pH meter 211-02 (Hanna Instruments, Germany). Basic ions were measured by ion chromatography using conductometric analysis by the LC–20 Prominence (Shimadzu) liquid chromatograph. HCO_3 weight concentration was determined by means of the potentiometric titration method using a pH meter 211-02 (Hanna Instruments, Germany).

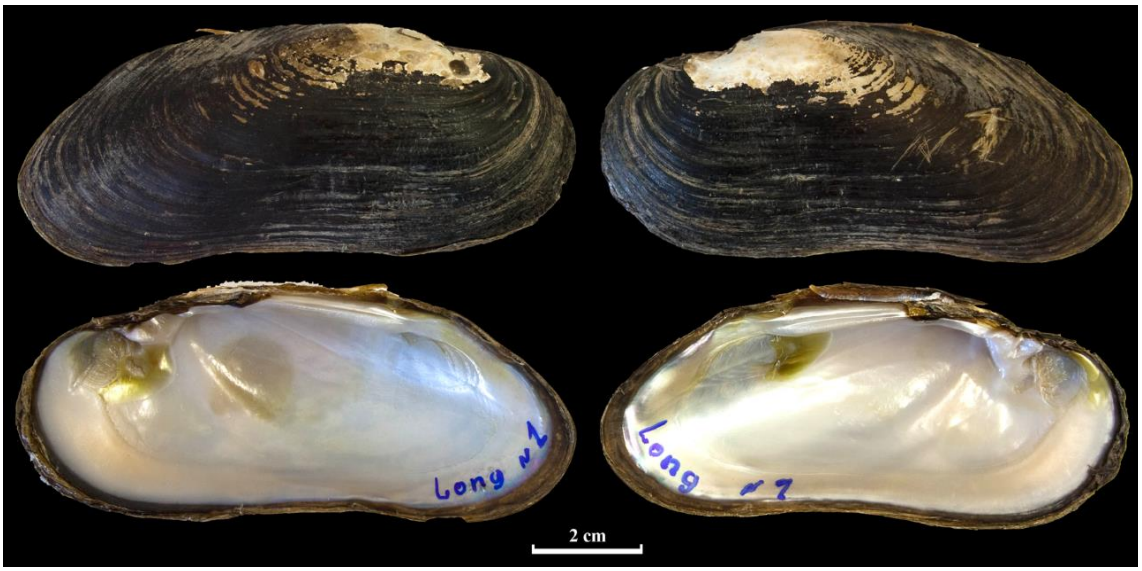

Fig. 3. Shell of *Margaritifera laosensis* specimen from the river Nam Long (Catalogue No. Mekong–La–1).

Results

Margaritifera laosensis was detected in two of 10 tributaries of the Nam Ou River. Specimens were found only in the Nam Long and Nam Pe rivers, two mountainous, oligotrophic headwaters with circumneutral pH (Table 1). Populations were restricted to reaches at altitudes of 460 to 890 m (elevation of the surrounding mountain ranges is 1,400–1,800 m). Calcium concentration in both streams was ~30 mg/l (Table 1). Occupied mesohabitats included riffles and runs with relatively coarse substrates, including gravel and fine gravels associated with boulders and numerous bedrock outcroppings (Appendix 1). Few macrophytes were observed in streams

supporting *M. laosensis* populations, but substrates frequently supported algal growth. Median depth and velocity values for occupied sites were 0.2 m and 0.3 ms^{-1}, respectively.

Table 1. Physico-chemical analyses of the water at the sample sites of the Nam Ou River tributaries at low water level (4 – 13 May 2012)*

Parameters	Mean ± *SD*	min - max
pH	7.7±0.3	7.1-8.3
Temperature (ºC)	26.8±1.6	25.4-30.3
Ca (mg/l)	32.3±4.0	26.9-37.6
Mg (mg/l)	7.3±1.7	4.4-9.7
Na (mg/l)	8.1±2.4	4.6-11.9
Cl (mg/l)	1.0±0.6	0.6-2.1
SO_4 (mg/l)	3.0±0.7	1.8-4.2
HCO_3 (mg/l)	16.9±3.2	12.0-21.0
NO_3 (mg/l)	0.08±0.14	0.00-0.42
NO_2 (mg/l)	0.05±0.05	0.00-0.14
PO_4 (mg/l)	0.00	-

* - *n* = 9; three samples from the river Nam Long and one sample from the rivers Nam Pe, Nam Ban, Nam H.Hu, Nam Houn, Nam Phet and Nam Phan. One sample from the Nam Leng River was excluded as an outlier due to extremely high mineralization.

Pearl mussel density is very low at all sites surveyed (Appendix 1). We located a total of 252 individuals from both streams, but only 78 (31.0 %) were alive (Fig. 2B); 79 individuals (31.3 %) had likely died after harvesting by local people (Fig. 2C) and 95 shells (37.7 %) probably died by other causes (e.g., post-flood stranding, mammal and bird predation, disease). The largest mussel population was found in the River Nam Long (66 individuals, 84.6%). The majority of individuals encountered were observed near the riverbank in the shadow of overhanging trees and bushes (67 individuals; 85.9 %). The remaining 11 mussels (14.1 %) were found in mid-channel habitats. Pearl mussels primarily occurred in coarse sand and fine gravel substrates, frequently in micro–grottos downstream from cobble and boulder substrates (58 individuals; 74.4 %). Twenty individuals (25.6 %) occupied sandy and fine gravel substrates between stones. Many of the mussels encountered (~50%) were almost completely burrowed beneath the substrate with only their siphons protruding. Sub-adults (<50 mm) were completely burrowed beneath the sand.

Morphological comparisons of collected specimens with type descriptions [35] suggest that *M. laosensis* may represent a distinct (and not multiple) taxon. Nacre color of all specimens was white, often with small oily spots (Fig. 3). The mean length of *M. laosensis* sampled was 65.5 mm (*SD* = 21.4 mm, range 26.1-110 mm, Figs. 4A, B). In the Nam Long River, the numerically dominant size class (66.7 %) of living mussels were 31-70 mm long, and the numerically dominant size class of individuals (40.9 %) were 51–70 mm long (Fig. 4C). In the Nam Pe River, the numerically dominant size class (41.7 %) of mussels were 71–80 mm long (Fig. 4D). In the Nam Long River, collected mussels had died from natural causes; most of them were between

31 and 50 mm in length (48.3%). In Nam Pe, shells were harvested by villagers and the maximum frequency of dead mussels was in the large size class of 81–100 mm (49.4%).

The youngest mussels found alive were four years old with mean length 34.4 mm from the Nam Long River and 38.6 mm from the Nam Pe River. A 36.8-mm empty shell of the same approximate age was found in the Nam Long River. Only six other specimens <55 mm total length were found during this survey. Ages of three largest shells (99.6-110 mm) from the Nam Long River ranged from 11-12 years and the three largest shells from the Nam Pe River (97.5-105.8 mm) were aged 10 and 11 years.

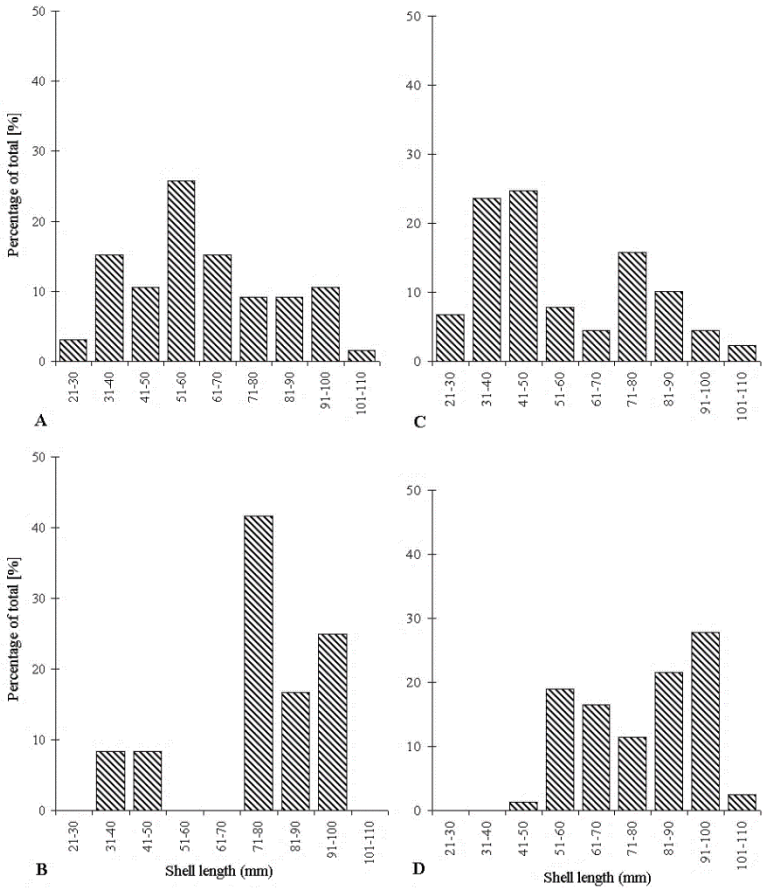

Fig. 4. The size frequency structure of live mussels and dead shell samples observed in the study area. A – river Nam Long (live mussels; sites NL1–NL5; *n* = 66); B – river Nam Pe (live mussels; sites NP1 and NP2; *n* = 12); C – river Nam Long (shells of mussels, died from natural causes; sites NL1–NL5; *n* = 89); D – river Nam Pe (shells of mussels, harvested by local people; sites NP1 and NP2; *n* = 79). Differences between all groups are significant by Kruskal-Wallis test: χ^2 (*H*) = 51.5, *df* = 3, *n* = 246, *p* < 0.001.

Discussion

Recently, *M. laosensis* was recorded in the Nam Long River and the Gnot Ou River [8, 9], and is now first found in the Nam Pe River, tributary of the River Nam Ou. These are the only known viable populations of this species in the world.

Margaritifera laosensis occupied tropical mountain river habitats with fast flow, coarse substrates, and low in dissolved solids and organic matter concentrations. These oligotrophic rivers are uncommon in this region and may explain the widespread but scattered distribution of this mussel on the Indochinese Peninsula. *Margaritifera laosensis* has substrate and depth preferences similar to other Margaritiferidae, including the Holarctic *M. margaritifera* [30, 36-

38]. However, the majority of live pearl mussels in both of our study populations were observed near the riverbank in the shadow of overhanging trees and bushes. This microhabitat preference is mirrored by *M. margaritifera* populations in several European rivers [38-42]. Large European pearl mussel populations may be found across the river bed, but small populations in nutrient-enriched or physically disturbed streams are often found close to stream banks [43]. Occurrence of live individuals along river banks and in micro-cavities behind large stones may represent an adaptation to rapidly changing hydrological conditions typical of many Southeast Asian streams.

River ecology is dominated by flow seasonality imposed by monsoonal rains [43]. Declines in zoobenthos abundance occur during the wet season in many small streams, as spates may initiate catastrophic drift and washout [44]. Riffles are the most stable mesohabitats in many rivers, because their coarse, armored substrates are the least disturbed by floods [45]. Flood size and frequency are important factors limiting populations of European *M. margaritifera,* and huge floods destroyed several large mussel beds in the rivers of Scotland, UK [46, 47].

Interestingly, many water chemistry parameters of Nam Ou Drainage streams are similar to European pearl mussel streams. However, Nam Ou Drainage streams have much higher Ca^{2+} concentrations (26.9–37.6 mg/l) than European *M. margaritifera* streams (typically <10 mg/l) [24, 25, 48, 49]. *Margaritifera margaritifera* may inhabit streams with intermediate and high calcium concentrations (e.g., 20–90 mg/l) in limestone rich regions of Ireland and Wales, UK, but this is atypical [46, 47].

Margaritifera laosensis in our study streams are younger and thus appear to grow faster than do European congeners [51, 52]. However, visual evidence for winter growth stops in *M. laosensis* was not clearly defined, likely because winters are typically mild in this region.

Shell length was also weakly correlated with apparent mussel age. In the Nam Long River, the largest shells were not necessarily the oldest. We have no data on age to maturity of Indochinese pearl mussels. Natural mortality in *M. laosensis* is mainly associated with smaller-sized mussels (<50 mm in length); however, villagers harvested larger specimens (>80 mm in length).

The presence of live small-sized mussels in the Nam Long and Nam Pe rivers suggests recent recruitment in these populations. Host fishes for Indochinese *Margaritifera* glochidia are unknown, but total fish diversity in the Nam Ou drainage is estimated at 72–84 species [8, 53].

Implications for conservation

Threats to Indochinese pearl shell mussel populations in the Nam Ou Drainage include three main issues. First, *M. laosensis* is a traditional food harvested by rural villagers in the Phongsali Province of Laos. Villagers also harvest and sell thiarid gastropods and corbiculid bivalves. Villagers harvest *Margaritifera* incidentally when foraging for other freshwater species, typically during the dry season (Fig. 2C). Second, river regulation and dam construction threaten Indochinese pearl mussels. Simple dams made of stones, trunks or bamboo stakes are frequently constructed by villagers to increase water levels by 0.3–1.0 m. The upper Nam Pe River has three such primitive dams and the Nam Long River has two dams. Impounded reaches upstream from these dams contain thick layers of decomposing plant litter, and *M. laosensis* were not found in the dammed areas of rivers. Finally, deforestation and agriculture are leading to increased water pollution in this region. Deforestation is widespread in the Nam Long and Nam Pe River Basins, but is primarily associated with slash-and-burn agriculture. The most widespread types of land cover include evergreen shrubs and fragmented or regenerating forests (~80 % of total area in our study watershed). Steep, devegetated mountain slopes and river valleys lacking plant cover

experience intense erosion during monsoon rains. As a result, many streams are highly impacted by clay and other fine sediments. Interestingly, N and P concentrations remain very low in rivers because fertilizer use is uncommon and most villages and cattle farms in the region are small or not located close to our study rivers (Table 1).

We believe that the most significant threat to Indochinese pearl mussel populations comes from harvest by local people as well as from expansion of agricultural land into stream riparian zones [13].

As has been advocated for other Margaritiferidae bivalves, an integrative conservation strategy that identifies and sustains ecological processes and evolutionary lineages is urgently needed to protect and manage this imperiled species [41]. The main conservation priorities for the Indochinese pearl mussel in the Nam Pe and Nam Long rivers include: 1) habitat conservation, including establishment of nature reserves within each river basin, 2) imposing a legislative ban on Indochinese pearl mussel harvest in Laos and neighbouring countries (Myanmar and Vietnam), 3) inventorying the distribution and current status of populations in Northern Laos (Nam Ou River Basin) and Northern Myanmar (Salween River Basin), and 4) identifying the glochidia host fish and describing key life history traits.

The above conservation measures may not only restore mussel populations, but also positively affect water quality and habitat of the entire upper Nam Ou basin. Livelihoods of the local communities of the region are linked with water resources: water quality in small rivers, water level in the Nam Ou, and fish resources. Pearl mussels, as “umbrella species,” construct habitat for their own and for other water organisms, and increase water quality. A basin approach for new protected areas, especially conservation of forests within river basins, will positively influence the hydrology of the region. These changes will bring positive effects to local communities: more clear water for home needs, longer navigation period due to increase of high water level period, and increased fish catch.

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Appendix 1. Habitats and density of *Margaritifera laosensis* populations in the rivers Nam Long and Nam Pe

River	Part of river	Site code	Site altitude, m. a.s.l.	River width, m**	Mesohabitat type	Dominant substrate type	Depth, m**	Current velocity, ms^{-1}	Square of observation site, m^2	*Margaritifera* population	
										Total ind. observed	Density, ind./m^2
Nam Long	downstream	NL1	480	12–15	Riffle	gravels	0.05–0.2	0.2	132	27	0.2
--//--	--//--	NL2	480	--//--	--//--	--//--	0.05–0.1	0.6	14	18	1.3
--//--	--//--	NL3	500	--//--	--//--	gravels and fine gravels	0.2–0.5	0.3	240	14	0.06
--//--	--//--	NL4	500	--//--	--//--	gravels	0.2–0.6	0.3	276	4	0.01
--//--	--//--	NL5	520	--//--	Run	--//--	0.3–1.5	0.2	320	3	0.01
Nam Pe	downstream	NP1	460	15–20	Riffle	gravels and fine gravels	0.1–0.3	0.3	70	10/69*	0.14/1.0*
--//--	upstream	NP2	890	10–15	--//--	gravels	0.1–0.6	0.3–1.8	355	2/22*	0.01/0.06*

* – The numerator contains the amount of specimens and evaluated values of compactness according to our sampling, the denominator represents the sum of mussel specimens found by us and the number of specimens harvested on this site by locals (for food); this value was estimated by means of calculation of open and left fresh shells with mussel's body remainders.

** – Width and depth of water streams were evaluated when water levels were low, May 2012. During rainy season water streams double their width (and more, depending on shore type).