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Unlocking the Secrets of Sturgeon Ecology: Lessons From Conservation Efforts in Northern Italy

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ABSTRACT

This study aimed to investigate the ecology of the Adriatic sturgeon (*Acipenser naccarii*) and Beluga sturgeon (*Huso huso*) in the Po basin in northern Italy, where both species have faced significant population declines. Using data collected from three LIFE projects, we investigated movement behaviour and habitat selection of the species following restocking, reintroduction and river defragmentation actions implemented during the LIFE projects. The two species showed a distinct movement behaviour and habitat selection. *A. naccarii* preferred to stay in pool, and it showed a tendency to have a landlocked behaviour, whereas *H. huso* did not show a clear habitat selection, and it always moved downstream, supporting the typical anadromous behaviour of this species. Both species benefited from river defragmentation interventions. These findings underscore the importance of habitat conservation/restoration and defragmentation measures for the long-term survival of sturgeon species.

1 | Introduction

Since the 1980s, the Acipenseridae family has faced a significant decline worldwide due to overfishing, habitat degradation and river fragmentation (Billard and Leconte 2000). In Italy, a similar trend occurred during the 20th century. Overfishing led to a drastic reduction of population consistency of the three species inhabiting national seas and rivers (Rossi et al. 1991), namely, the Adriatic sturgeon (*Acipenser naccarii*), the European sturgeon (*Acipenser sturio*) and the Beluga sturgeon (*Huso huso*). Despite a nationwide fishing ban imposed on these species in 1980, illegal fishing has continued to pose a significant threat to sturgeon populations. In the 1990s, *A. sturio* and *H. huso* were officially recognized as locally extinct in Italy. This resulted in

A. naccarii remaining the only surviving sturgeon species in the country (Rossi et al. 1991) until the *H. huso* reintroduction in 2019.

A. naccarii is an endemic species of the Adriatic Sea basin and was historically present in the northern Italian rivers and in the rivers of Albania (Bronzi, Vecsei, and Arlati 2005) and Greece (Paschos et al. 2003). Its range is currently reduced to the upper Adriatic Sea and the Po River and its main tributaries. The contraction of the population's range was mainly caused by overfishing of prereproductive size individuals and rivers' fragmentation (Birstein, Bemis, and Waldman 1997; Vecsei, Sucui, and Peterson 2002; Bronzi, Congiu, and Freyhof 2022). This anadromous species may form landlocked populations in case

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of dams (Kottelat and Freyhof 2007). One landlocked population of *A. naccarii* has been found in the Ticino River, one of the main tributaries of the Po River. Currently, the Italian population seems to have an increasing trend (Bronzi, Congiu, and Freyhof 2022).

H. huso is an anadromous fish whose surviving populations are limited to the Black Sea, the Caspian Sea and Sea of Azov and their tributary rivers, whereas historically, it also occurred in the Adriatic Sea (Vecsei, Sucui, and Peterson 2002; Kottelat and Freyhof 2007). It typically migrates upstream to spawn in large rivers during spring. Juveniles then migrate downstream to marine environments, where they grow up and spend most of their adult lives. Upon reaching sexual maturity, typically between 12 and 22 years of age depending on sex, every 4–7 years, they return to fresh waters to spawn (Vecsei, Sucui, and Peterson 2002). Between the nineteenth and twentieth century, the Adriatic population occupied an area spanning from the Po River Delta to the lower course of its tributaries. However, after a rapid decline, *H. huso* became locally extinct in Italy during the 1970s. The negative trend of the populations of Eastern Europe leads the species to be classified as Critically Endangered by the IUCN Red List of Threatened Species (Gessner, Chebanov, and Freyhof 2022).

Despite the ecological and conservation importance of these species, neither historical nor recent exhaustive studies on the ecology of Italian sturgeon populations are available. In the last 20 years, important advances have been made for the conservation of these two species through three European LIFE projects. The LIFE programme is the EU's funding instrument for environment and climate action. Specifically, the Nature and Biodiversity subprogramme aims at the protection and restoration of Europe's nature and halting and reversing biodiversity loss in European countries. Data collected and outputs from these projects can certainly represent a valuable source of information, especially for endangered species or for those species whose survey and monitoring are highly demanding. Indeed, such projects can rely on a considerable involvement of people, local associations, administrations and on significant funding. However, too often, the results from these projects remain as 'grey literature' unavailable for further research.

The first project, named 'LIFE ACIPENSER TICINO-LOMB' (LIFE03 NAT/IT/000113; <https://webgate.ec.europa.eu/life/publicWebsite/project/LIFE03-NAT-IT-000113/conservation-of-acipenser-naccarii-in-the-river-ticino-and-in-the-middle-reach-of-the-river-po>) was carried out between 2003 and 2006 and aimed to increase knowledge of the landlocked *A. naccarii* population of the Ticino River and to develop an action plan for its long-term conservation (Puzzi et al. 2009). Some captive-reared individuals were released in the Ticino River and monitored with acoustic transmitters and boat-operated mobile hydrophones. Data from this monitoring, integrated with data from previous fish censuses, showed that the final stretch of the Ticino River was the most inhabited by *A. naccarii*.

The second LIFE project (2012–2017), called 'LIFE CON.FLU.PO.' (LIFE11 NAT/IT/000188; <https://webgate.ec.europa.eu/life/publicWebsite/project/LIFE11-NAT-IT-000188/restoring-connectivity-in-po-river-basin-opening-migratory-route-for-acipenser-naccarii-and-10-fish-species-in-annex-ii>) aimed at reducing fluvial habitat fragmentation along the Po River, enhance hydrological connectivity and conserve native fish species of the Po River basin.

Therefore, an infrastructure allowing fish upstream passage was realized at the Isola Serafini dam (Po River, Monticelli d'Ongina, Province of Piacenza), one of the main ecological barriers along the Po River catchment, and a restocking of populations of *A. naccarii* and other native fish species was carried out. Along this project, some reared *A. naccarii* individuals were tagged and released in both the Ticino and Po rivers and were monitored with fixed and mobile receivers to verify the effectiveness of the defragmentation action.

The last project, named 'LIFE TicinoBiosource' (LIFE15 NAT/IT/000989; <https://webgate.ec.europa.eu/life/publicWebsite/project/LIFE15-NAT-IT-000989/enhancing-biodiversity-by-restoring-source-areas-for-priority-and-other-species-of-community-interest-in-ticino-park>) was implemented between 2017 and 2021 and aimed at preserving and restoring several habitats and species of conservation interest in the territory of the Regional Park of Ticino Valley, in Lombardy (northern Italy), a protected area with probably the highest conservation value for wildlife and habitats across the whole Po Plain. Among the diverse project actions, one of them led to the creation of a new SAC (Special Area of Conservation, Natura 2000 Network: IT2080026; https://natura2000.eea.europa.eu/?views=Sites_View) called 'Breeding Sites of *Acipenser naccarii*' in 2020, protecting a stretch of the Ticino River known as the unique site where the species spawns. Furthermore, a rearing activity of *H. huso* was launched with the purpose of species reintroduction in the Ticino River. The released individuals were marked with acoustic transmitters and monitored with fixed and mobile receivers.

In this study, we pooled data collected through these three EU LIFE projects about *A. naccarii* and *H. huso* to investigate movement behaviour and mesohabitat selection of the species in a river catchment in northern Italy. We hypothesized that a part of the individuals of *A. naccarii* will remain in the freshwater without reaching the sea, considering its potential to form landlocked populations; conversely, we hypothesized that individuals of *H. huso* will migrate downstream towards the Po Delta and the sea. Concerning mesohabitat selection, no prior hypotheses were formulated considering that very little is known on their habitat preferences in Italian populations.

2 | Methods

2.1 | Fish and Telemetry Data

Within the EU LIFE projects, between 2005 and 2020, 140 *A. naccarii* and 25 *H. huso* individuals (all subadults for both species) were tagged with ultrasound acoustic transmitters and released in the Po basin. In the 'LIFE ACIPENSER TICINO-LOMB', acoustic transmitters Sonotronics CTT-83-3-I (62 mm length × 16 mm diameter, 20-g weight) and mobile receivers Sonotronics MANTRAK were used. In the 'LIFE CON.FLU.PO.' and 'LIFE TicinoBiosource' acoustic

transmitters Lotek MM-MR-11-45 (73 mm length × 12 mm diameter, 14.5-g weight) and receivers Lotek WHS 6000 were used. Tags were inserted into an abdominal incision (5- to 6-cm length) closed through a suture after the application of a disinfectant (povidone-iodine). To reduce any stress and risk to the captured individuals, sedation was not applied because the individuals appeared relaxed during tagging operations. Captured individuals were placed into an artificial pool for 20 days, and their conditions were monitored to verify the healing of the injury and that the transmitter had not been accidentally lost. Tag mortality was essentially null (only one individual died after the surgery). Individuals were weighed and measured (length) after the capture.

A. naccarii individuals were released both in the middle-lower Ticino River, between Cassolnovo and Pavia (spring 2005, 2006 and spring and summer 2017), and in the Po River, in Piacenza and Rovigo (spring and summer 2017). *H. huso* specimens were released only in the middle-lower Ticino River (spring 2019 and January 2020). Monitoring was conducted exclusively by mobile hydrophones in the first project ('LIFE ACIPENSER TICINO-LOMB'), by three fixed receivers and opportunistic sightings in the second project ('LIFE CON.FLU.PO.'), and by four fixed receivers and mobile hydrophones in the third project ('LIFE TicinoBiosource'). Fifty-eight individuals of *A. naccarii* and 18 of *H. huso* were detected by receivers and analysed for the movement analysis.

2.2 | Movement Analysis

Receivers were checked monthly, and descriptive fish movements were summarized by using every single individual detection over the whole period (2005–2020). Specifically, we calculated (i) the total displacement, that is, the sum of the length of each section travelled between the release and the first survey point and between one survey point and the next one; (ii) the maximum displacement, that is, the distance between the release point and the farthest detection point from it; and (iii) the movement direction by assessing whether the sturgeons (a) remained in the river where they were released, (b) reached a tributary or an emissary or (c) reached the sea.

2.3 | Mesohabitat Selection Analysis

We used data collected from the 'LIFE ACIPENSER TICINO-LOMB' in the middle-lower Ticino River between Vigevano and Pavia, because in this section, the whole river stretch was evenly investigated by a mobile hydrophone. Three types of mesohabitats were assigned within the surveyed river stretch: *pool* (i.e., hydrological unit characterized by a weaker flow, topographic depression and inverse slope in the downstream section), *riffle* (i.e., hydrological unit characterized by shallow water and turbulent flow with unbroken standing waves with crests facing upstream and moving in a downstream direction) and *run* (i.e., hydrological unit often placed between *pool* and *riffle* and characterized by a minor slope than riffle, weak turbulence that does not produce waves but symmetrical ripples that move in a downstream direction) (Jowett 1993; Zavadil et al. 2012; Vezza, Zanin, and Parasiewicz 2017).

To assess the habitat selection of the two species, a *use versus availability* approach was used. Detection points of marked *A. naccarii* were used as presence points (155 presence points on 41 individuals for *A. naccarii* and 10 presence points on eight individuals for *H. huso*). Considering that we have presence-only data, in order to adequately represent the range of environmental conditions in the study area, a number of pseudoabsences (i.e., artificial absence data; Phillips et al. 2009) equal to four times the number of presence points for each species was randomly generated along the investigated river stretch. Each point was associated with the corresponding type of mesohabitat.

For both species, a generalized linear model (GLM) with a binomial error distribution was performed to assess the effect of the mesohabitat on species' occurrence. Considering that the application of a generalized linear mixed model with a random intercept for the individual ID was not feasible due to fact that the majority of case (26 out of 41 of the total individuals for *A. naccarii* and all the individuals for *H. huso*) counted less than three detections, we grouped the occurrence by the individual ID and assigned as mesohabitat the main type used by the individual (i.e., > 50% of the total number of detection were fallen within that specific category) and maintained a correspondent proportion of pseudoabsences across the three types of mesohabitat. For both species, we tested the significance between all possible comparisons of each pair of mesohabitats using the Tukey method for the *p* value adjustments for multiple comparisons using the R package *emmeans* (Lenth 2024). All the analyses were conducted in the R software v. 4.3.2 (R Core Team 2021).

3 | Results

3.1 | Movement Analysis

Of the 58 *A. naccarii* individuals marked, 41 were released in the Ticino River and 17 in the Po River. An average total displacement of 8.7 km (standard deviation = 8.4 km) was detected for the 41 *A. naccarii* released in the Ticino River. The maximum displacement detected was 25.6 km. Eleven individuals (26.8%) did not move from the release point (displacement less than 1 km), whereas 19 individuals (46.3%) moved downstream and 11 (26.8%) upstream, but 100% of the individuals remained in the Ticino River (Figure 1). Conversely, the 17 individuals of *A. naccarii* released in the Po River showed a larger displacement, amounting to 40.3 km (standard deviation = 59.5 km). The highest detected displacement was 254.8 km. Twelve individuals (70.6%) moved downstream and reached the Po Delta, four of them (23.5%) moved upstream but remaining in the Po River and only one (5.9%) moved upstream and reached the Ticino River (Figure 1).

Eighteen *H. huso* individuals were released in the Ticino River. An average displacement of 107.1 km (standard deviation = 155 km) was detected. The largest detected displacement was 501.1 km. All the released individuals descended downstream the river, whose 33.3% (six individuals) remained in the Ticino River, 50% (nine individuals) reached the Po River and 16.7% (three individuals) reached the Po Delta and the Adriatic Sea (Figure 2).



Legend

- Ticino and Po Rivers
- Release point
- △ Fixed receivers LIFE11 NAT/IT/000188 and LIFE15 NAT/IT/000989
- ▲ Fixed receivers LIFE15 NAT/IT/000989
- //// Localised displacement between Cassolnovo and Pavia
- Displacement upstream from Piacenza to Travacò Siccomario
- Displacement downstream from Piacenza to Papozze
- Displacement upstream from Rovigo to S. Maria Maddalena
- Displacement downstream from Rovigo to Papozze

FIGURE 1 | Movements of tagged *Acipenser naccarii* in the Po basin (2005–2017).

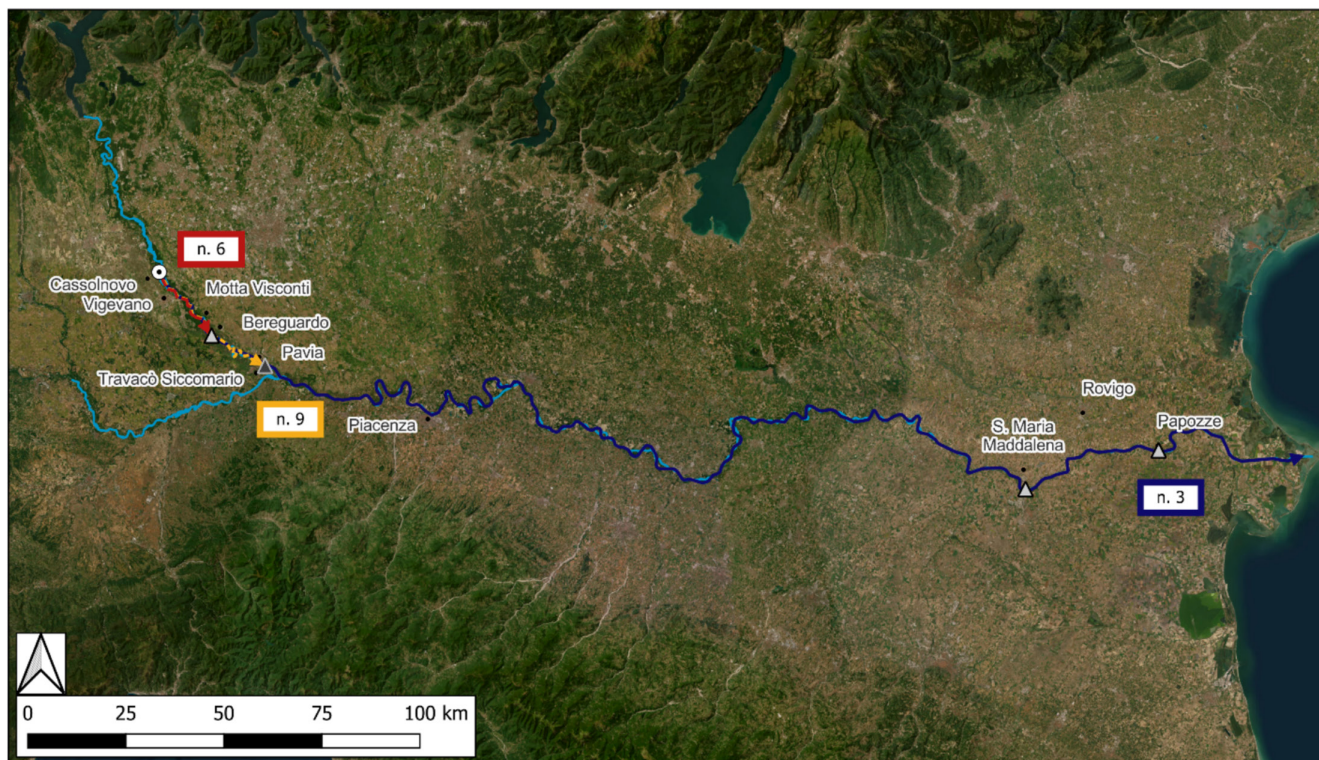
3.2 | Mesohabitat Selection Analysis

A. naccarii showed a significant preference for the use of *pools* over all the other two mesohabitat categories, but no difference was detected for the comparison between *riffle* and *run* (Table 1). The adjusted *R* squared was 0.243. Conversely, *H. huso* did not seem to have marked mesohabitat preferences, although *runs* showed a tendency to be avoided (close to the statistical significance threshold) compared to *pools* (Table 1). The adjusted *R* squared was 0.204, albeit the results should be considered cautiously as the low sample size ($N=40$).

4 | Discussion

The results showed that *A. naccarii* and *H. huso* inhabiting the Po River basin are characterized by a different ecology. *A. naccarii* seems to have a lower tendency for movement and a duality of behaviour depending on its release location. The stock released in the Po River moved mainly towards the sea. In contrast, the individuals released in the Ticino River mainly moved across an area limited to the release site, and, despite some individuals traversing several kilometres, all of them remained within the Ticino River. Interestingly, these individuals behaved like a landlocked population, but this evidence cannot be attributed to *homing* behaviour, as the animals were born and raised *ex situ* and were released only

at subadult age. *A. naccarii* probably found optimal environmental conditions in the Ticino River, which led the species to carrying out its entire life cycle within the freshwater ecosystem. The finding of juveniles in the river stretches between Pavia and Travacò Siccomario (Figure 1) between 2009 and 2015 suggests that *A. naccarii* can complete the entire life cycle in the Ticino River and survive as landlocked populations in rivers (Ludwig et al. 2003; Zerunian 2003). Our findings suggested the importance of the *pool* mesohabitat to preserve the species in the Ticino River; some monitored individuals continuously frequented the same pools for more than a year after the release. This preference for *pools* might be linked to a higher availability of favourite food resources (mainly crustaceans gammarids, dipteran chironomids and oligochaetes in Ticino and Po Rivers; Zerunian 2003) in this type of mesohabitat. This finding underscores the importance of preserving or creating pool mesohabitats year-round. Instream management and restoration should prioritize actions that maintain or establish new pools. For instance, measures might include implementing suitable flow regulation in river catchments to ensure a minimum flow, promoting the persistence of sufficiently deep pools during drought periods. Additionally, conserving or enhancing stream complexity within the channel morphology can facilitate the formation of new pools, such as through the placement of underwater wood logs in the channel. The significance of these pools may be especially pronounced during the reproduction season, as the species prefer weaker water flow for spawning (Zerunian 2003).



Legend

- Ticino and Po Rivers
- Displacement downstream in the Ticino River
- Displacement downstream to the Po River (Travacò Siccomario)
- Displacement downstream to the Po Delta
- ⊙ Release point
- △ Fixed receivers LIFE11 NAT/IT/000188 and LIFE15 NAT/IT/000989
- ▲ Fixed receivers LIFE15 NAT/IT/000989

FIGURE 2 | Movements of tagged *Huso huso* in the Po basin (2019–2020).

TABLE 1 | Comparison between the differences of the estimated coefficients through the GLMs between the three mesohabitats.

	Mesohabitat type	Estimate	SE	z value	P
<i>Acipenser naccarii</i>	Pool—riffle	3.209	0.816	3.932	< 0.001
	Pool—run	2.601	0.442	5.881	< 0.001
	Riffle—run	−0.608	0.777	−0.782	0.714
<i>Huso huso</i>	Pool—riffle	18.859	2465	0.008	1.000
	Pool—run	1.992	0.937	2.125	0.085
	Riffle—run	−16.866	2465	−0.007	1.000

Note: *Acipenser naccarii*, $N = 205$; pool, $N = 34$; riffle, $N = 29$; run, $N = 142$; *Huso huso*, $N = 40$; pool, $N = 7$; riffle, $N = 7$; run, $N = 26$.

Abbreviation: SE, standard error of estimates; z, Wald statistic for testing the hypothesis that the corresponding estimate is equal to zero (null hypothesis).

Conversely, all released individuals of *H. huso* covered greater distances, moving downstream, sometimes reaching the sea. This behaviour can be attributed to the species biology. *H. huso* is a strict anadromous species (Zerunian 2003). Habitat selection model for *H. huso* showed no significant differences among the mesohabitats (Table 1). This might be due to individuals tending to out-migrate from the release point to reach the sea. All tagged *H. huso* individuals were subadults, and considering the species' pronounced anadromous behaviour, they likely aim to reach the sea for growth. However, the lack of significance could be influenced by the small sample size, and we cannot exclude the importance of some mesohabitats during different phases of the

life cycle. It is known that this species prefers highly oxygenated water with strong current and stone/gravel bottom for spawning (Kottelat and Freyhof 2007); hence, this habitat could be crucial for a successful reproduction in these rivers following river defragmentation. The effectiveness of reintroductions and the survival of *H. huso* depends on its ability to fulfil its reproductive cycle through migrations between rivers and sea, making river corridor defragmentation actions such as that of Isola Serafini implemented through the 'LIFE CON.FLU.PO.' being crucial for the species conservation. Also, defragmentation of river corridors is certainly important for the long-term conservation of *A. naccarii* to restore a long-term functional gene flow with other

Adriatic populations. Considering the role as umbrella species for *A. naccarii* and *H. huso*, the implemented defragmentation measures for these two species could promote benefits to the whole freshwater fish community (Carrizo et al. 2007; Kalinkat et al. 2017; Rachler et al. 2017).

This research started to shed light on movement ecology and behaviour in Italian populations of sturgeon species, for which no targeted studies were previously available. A first effort focused on the investigation of species movement behaviour and mesoscale habitat selection lays the groundwork for future studies aimed to sustain effective conservation actions for *A. naccarii* and to successfully pursue the reintroduction of *H. huso* in Italy or in other countries. In our study, the displacement was assessed using a descriptive approach, which is limited to evaluating spatial movement patterns without analysing the causes of the observed movements. Indeed, understanding mechanistic processes is essential to implement effective management strategies for endangered and poorly known species of sturgeons. Investigating their ecological needs across the full life cycle is also pivotal to guaranteeing the success of conservation actions. Therefore, the application of probabilistic and mechanistic models (e.g., multistate models) to explain how individual movements are influenced by environmental characteristics and seasonality, and to estimate movement rates, survival and detection probabilities, is recommended. This approach can provide a deep understanding of the ecology of these species as well as valuable behavioural insights for their conservation. We stress the importance of valuing data collected during large, well-funded conservation projects, such as the EU LIFE programmes, as these data can be very important to support evidence-based conservation actions. Hence, it is advisable to carefully plan all activities (e.g., sampling, monitoring, data collection and curation) while also considering the conceptualization of research questions and methodological framework that allows to achieve key findings to support the conservation of these species, therefore maximizing the huge efforts involved in these projects.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Considering that the species under study are of conservation concern, the georeferenced data supporting the findings of this study are available upon reasonable request from the corresponding author.

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